Remarks

The Applicants acknowledge the provisional rejection of Claims 11 and 27 over Application Nos. 09/436,265; 09/939,483; 09/939,484; and 09/892,360. The Applicants respectfully request that further treatment of the rejection be held in abeyance. Support for the Applicants' amendment to Claim 27 can be found on page 6, line 17, to page 9, line 2. The Applicants have added new Claim 28, which depends from Claim 27. Support for new claim 28 can be found in Figs. 1 and 2. New Claim 28, is drawn to the subject matter of the elected invention. No new matter has been added. Thus, Claims 11, 27, and 28 are pending for the Examiner's consideration.

Claim Rejections under 35 U.S.C. § 101

Claims 11 and 27 have been rejected under 35 U.S.C. § 101. 35 U.S.C. § 101 prescribes that "whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter... may obtain a patent thereof..."

Thus, § 101 is designed to grant patents to those inventions which are "useful." An invention is useful if it has a specific, substantial and credible utility. A "specific utility" is a utility that is specific to the subject matter claimed. A "substantial utility" defines a "real world" use. An asserted utility is "credible" if it is believable based on the record and the nature of the invention. To refute the establishment of "credible utility", the Examiner must demonstrate that it is more likely than not that one of ordinary skill in the art would doubt the truth of the statement of utility. MPEP § 2107.02. Moreover, a specific, substantial and credible utility is established when the invention discloses sufficient information about the invention to make its usefulness immediately apparent to those familiar with the technological field of the invention.

MPEP § 2107.01 citing to Brenner v. Mason, 383 U.S. 519, 148 USPQ 689 (1966). Therefore, when the claimed invention has a specific, substantial and credible utility that is immediately apparent to those familiar with the art, a rejection based on lack of utility is improper. See MPEP § 2107.01.

In the instant case, a specific substantial and credible utility has been established for the claimed TWIK-1 potassium channel. In particular, the Applicants have disclosed a potassium channel protein having specific biological activity mediated by pharmacological and chemical agents *in vitro,* demonstrating the channel's therapeutic utility. The Examiner's attention is invited to the following passage from 2107.03 of the MPEP, which states that:

> The Federal Courts have consistently reversed rejections by the Office asserting a lack of utility for inventions claiming pharmacological or therapeutic utility where an applicant has provided evidence that reasonably supports such a utility. In view of this, Office personnel should be particularly careful in their review of evidence provided in support of an asserted therapeutic or pharmacological utility.

Here, the Applicants' Specification demonstrates multiple therapeutic activities for the claimed potassium channel protein (TWIK-1). The Applicants have particularly described the structure (two pore domains and four transmembrane segments), function (inwardly rectifying K+ transport), locus (chromosome 1 q42-q43), tissue distribution (brain and heart), and regulation *in vitro* (transformed oocytes contacted with pharmacological agents) of a novel potassium channel. This novel potassium channel has utility at least as a new drug target for diseases involving K+ channels, particularly those diseases described on page 15 of the Applicants' Specification. Such diseases include neurodegenerative disorders, arrhythmia, epilepsy, anoxia, and ischemia. Consequently, the Applicants respectfully submit that the Specification has satisfied the requirements set forth in MPEP § 2107.03 and identified multiple

biological activities for the Applicants' claimed K+ channel protein that has a clear therapeutic utility which would be immediately appreciated by those familiar with the art.

The Applicants respectfully submit that TWIK-1 is clearly associated with a number of biological activities that establish therapeutic utility, and that the therapeutic utility of the claimed channel protein would be immediately apparent to those familiar to the art from those activities. For example, the Applicants demonstrate that TWIK-1 is an active voltage-gated potassium transport channel. TWIK-1 conducts the flux of potassium ions through the membranes of cells, which results in the movement of action potential and cell signaling. Such, voltage-gated channels are responsible for a number of specific cellular activities including the propagation of nerve impulses and heart rate control, and epithelial electrolyte transport. The Applicants submit herewith a copy of an article by Lesage et al. entitled *Molecular and functional properties of two–pore domain potassium channels,* Am J. Physiol Renal Physiol: 279: F793-901, 2000. This review article indicates that, due to their tissue distribution and functional properties, TWIK-1 could be involved in the control of background K+ conductances in many cell types.

The Applicants also submit a copy of *Potassium Channels: Molecular Defects, Diseases and Therapeutic Opportunities*, by Shieh et al., Pharmacol Rev. 52: 57-593, 2000 (hereinafter Shieh), for the Examiner's consideration. Shieh reviews a number of references that predate the Applicant's priority date of 1996. These references discuss the role of K+ channels in dysfunction associated with the heart and nervous system. The Applicants respectfully submit that this article reviews the role which potassium channels play in physiological function, their association with molecular defects, their use as a target for therapeutics, and their stimulation and inhibition by certain compounds. The review notes that:

> Potassium channels play important roles in **vital cellular signaling processes** in both excitable and non-excitable cells. Over 50 human genes encoding various K+ channels have been cloned during the past decade, and precise biophysical properties, subunit stoichiometry, channel assembly, and modulation by second messenger and ligands have been elucidated to a large extent. Recent advances in genetic linkage analysis have greatly facilitated the identification of many **disease-producing loci, and naturally occurring mutations in various K+ channels have been identified in diseases such as long-QT syndrome, episodic ataxia/myokymia, familial convulsions, hearing and vestibular diseases, Bartter's Syndrome, and familial persistent hyperinsulinemic hypoglycemia of infancy**. In addition, changes in K+ channel function have been associated with **cardiac hypertrophy and failure, apoptosis and oncogenesis in various neurodegenerative and neuromuscular** disorders. Concurrent with this remarkable progress in our understanding of molecular diversity, structure, and function, a growing number of discoveries have linked K^+ channel gene mutations with various diseases. Such diseases of the **heart**, kidney, pancreas, and central **nervous system** involve either mutation(s) in K^+ channel gene(s) and/or altered regulation of K^+ channel function. (Emphasis added).

Table 1 of Shieh provides a summary of genetically linked diseases of the cardiac, neuronal, renal, and metabolic system involving potassium channels.

TWIK-1 is highly expressed in the brain and heart, as described in the Applicants' Specification. Along those lines, Shieh recognizes:

> K1 channels are critical to cardiac excitability because they play a fundamental role in repolarization of the action potential. Unlike the action potentials of nerves that last only a few milliseconds, the action potentials of ventricular myocytes can last several hundred milliseconds. This prolonged depolarization phase is essential for normal excitation-contraction coupling process and renders the myocytes relatively refractory to premature excitation. Various classes of K1 channels with different time and voltage dependencies and pharmacological properties function in concert to regulate the heart rate by setting the resting membrane potential, amplitude, and duration of action potential and its refractoriness (Barry and Nerbonne, 1996). The Kir2.1 current sets the resting membrane potential and contributes to the terminal phase of repolarization. The transient outward K1 current (Kv4.3 or Kv1.4), which is Ca21-independent and expressed in a species- and cell type-specific fashion, is important for the early phase of repolarization. The long ventricular action potentials that result from the slow onset of repolarization are controlled mainly by two types of delayed rectifier K1 currents, i.e., IKs (derived from KCNQ1/minK) and IKr (derived from hERG/ MiRP1). Both genetic linkage analysis and the candidate gene approach revealed that

> mutations in these delayed rectifier K1 channel subunits form the molecular basis of LQT syndromes (Curran et al., 1995; Sanguinetti et al., 1995; Schott et al., 1995; Wang et al., 1996)
>
> K1 channels are critical to neurotransmission in the nervous system. Alterations in the function of these channels lead to remarkable perturbations in membrane excitability and neuronal function. Significant progress has been made in linking many neuronal disorders, including episodic ataxia and benign familial neonatal convulsions, to K1 channel mutations. (Ophoff et al., 1996, Litt et al. (1994).

Tables 3 and 4 of Shieh describe a large number of potassium channel inhibitors and openers. These potassium channel modulators can be used therapeutically in physiological systems ranging from cardiac, vascular, nonvascular muscle, neural, immune, and secretory systems. (See Table. 3 of Shieh). The Applicants respectfully submit that as of at least the priority date of the current Application, the art has clearly recognized the role of potassium channels in the heart and brain and their correlation to disease states in those organs. One skilled in the art as of the priority date of the present application would therefore, upon reading the Applicants' detailed description of TWIK-1 and its biological activity, would understand that TWIK-1 is at least a target for the development of therapeutic treatments. Thus, one familiar with the art would immediately appreciate the utility of the Applicants' claimed K+ channel.

The Applicants have therefore disclosed the therapeutic utility of the claim TWIK-1 protein, based on its demonstrated activity as an inward rectifying voltage-gated potassium channel. In particular, the Applicants' specification discloses on:

1) Page 9-10, that TWIK currents are inhibited by Ba^{2+}, quinine, quinidine, TEA, and Tedisamile, a Class III antiarrhythmia agent (e.g. TWIK's activity can be modulated by pharmacological agents and, as a result the flux of vital K+ ions can be mediated in cells expressing TWIK);

2) Page 16, that TWIK is expressed abundantly in the brain and heart (e.g. it is important for cardiac transport activity and neuronal regulation);

3) Page 15, that the TWIK gene was mapped to chromosome 1 q42-q43 (e.g. it allows for mutation/knockout studies and genetic therapy to transform mammals with mutated copies of TWIK);

4) Page 8, that TWIK demonstrated weak inward rectifying potassium currents (e.g. a potassium transport activity that is recognized by those skilled in the art: namely a role in involved in the control of background potassium membrane conductance);

5) Page 12, that TWIK potassium transport activity is indirectly effected by an acidic pH (e.g. pH can modulate activity); and

6) Page 1, that TWIK exhibits increased K+ currents when exposed to phorbol-12 myristate (PMA).

The Applicants have demonstrated the activity of TWIK-1 by expressing it *in vitro* in the oocytes of *X. laveis*. As discussed in the MPEP, *in vitro* data is sufficient to establish the therapeutic utility of a compound, composition or process. See MPEP §2107.3. MPEP § 2107.3 notes that courts have consistently found utility for a therapeutic invention despite the fact that an Applicant is at an early stage of development of a pharmaceutical product or therapeutic regimen, when it noted the significance of data from *in vitro* citing Cross v Iizuka, 224 USPQ 739 (Fed. Cir. 1985):

> We perceive no insurmountable difficulty, under appropriate circumstances, in finding that the first link in the screening chain, *in vitro* testing, may establish a practical utility for the compound in question. Successful *in vitro* testing will marshal resources and direct the expenditure of effort to further in vivo testing of the most potent compounds, thereby providing immediate benefit to the public.

In the present case, *in vitro* studies have revealed the significant biological role of TWIK-1. As a result, the Applicants respectfully submit that the Specification establishes therapeutic utility for TWIK-1 that would be immediately appreciated by those familiar with the art. The Applicants have shown that TWIK-1 potassium currents are highly sensitive to acidic pH, and inhibited by Ba^{2+}, quinine, quinidine, TEA and Tedisamil (an antiarrhythmia agent). The Applicants have also shown that TWIK-1 is not sensitive to K+ channel openers such as pinacidil and cromakaline (Fig 4 of Applicants' Specification). The Applicants have also described that TWIK-1's transcript is abundantly expressed in the brain and heart.

In sum, one skilled in the art would therefore recognize, from the disclosed biological activity of TWIK-1 that TWIK-1 could be used to:

1) target specific compounds to TWIK-1 to modify TWIK-1's action potential in localized tissue;
2) use the activity patterns of TWIK-1 to screen for therapeutic compounds, which inhibit or enhance TWIK-1 activity in localized tissue culture;
3) use TWIK-1's expression pattern, to evaluate and provide treatment for individuals suffering from heart and or neuronal disorders involving potassium transport;
4) use TWIK-1 for molecular diagnostics;
5) use genetic loci information for TWIK-1 to screen for TWIK-1 defects in people suffering from arrhythmia disorders; and
6) use TWIK-1 genetic loci (chromosome 1, q42-q43) to elucidate the role of TWIK-1 in pathophysiological and physiologic conditions.
7) as noted in page 15 of the Specification, use TWIK-1 to screen for drugs useful to treat heart and nervous system disorders including, epilepsy, arrhythmia, vascular disease, ischemia, anoxia, and endocrine diseases.

Thus, the Applicants have identified the biological role of TWIK-1 and its usefulness in therapeutics. The therapeutic utility derived from elucidation of the activity of the claimed TWIK-1 protein as described in the Specification clearly provides the public with an immediate benefit. Pharmacological or therapeutic related inventions that provide "**any immediate benefit to the public**" satisfy 35 U.S.C. §101. Nelson v. Bowler, 206 USPQ 881, 83 (CCPA 1980) (Emphasis added). In view of the foregoing, the Applicants respectfully request withdrawal of the rejection of Claims 11 and 27 under 35 U.S.C. §101.

Claim Rejections Under 35 U.S.C. § 112

Claims 11 and 27 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement or written description for any functional TWIK proteins except SEQ ID NO. 2. Applicants respectfully traverse these rejections.

To comply with the written description requirement, a specification must convey to one skilled in the art that the inventors, at the time of filing, were in possession of the claimed invention. To be enabling, a specification must teach one skilled in the art how to make and use the claimed invention without undue experimentation.

Here, claim 11 has been amended to recite that the claimed TWIK-1 protein is SEQ ID NO. 2. The primary amino acid sequence of SEQ ID NO. 2 is clearly set forth in the specification, and the nucleic acid sequence encoding SEQ ID NO. 2 is given in SEQ ID NO. 1. The working examples on pgs. 9-12 of the specification show that SEQ ID NO. 2 functions as a potassium channel.

One of ordinary skill in the art can readily produce a protein when provided with that protein's primary amino acid sequence, or when provided with the nucleic acid sequence encoding the protein, with no more than the application of routine chemical synthetic or molecular biological techniques. Moreover, the manner in which SEQ ID NO. 2 can be used is described in detail in the present working examples. The specification therefore conveys to one skilled in the art that the inventors were in possession of SEQ ID NO. 2, and provides ample guidance on how to make and use SEQ ID NO. 2 without undue experimentation. Claim 11 as amended is thus adequately described and enabled, and the Applicants respectfully request that the 35 U.S.C. § 112, first paragraph rejections of claim 11 be withdrawn.

Claim 27, as amended, is directed to functionally equivalent derivatives of SEQ ID NO. 2. Page 13, second paragraph of the specification characterizes such derivatives as proteins of SEQ ID NO. 2 in which one or more amino acids have been modified and/or suppressed. Claim 27 (and its dependent claim 28) define the claimed functionally equivalent derivatives as being suitable for transporting potassium across a membrane with weak inward rectification, and also

as comprising two pore domains P1 and P2, wherein said P2 domain comprises a GLG sequence; four transmembrane domains M1 to M4; an amino acid loop between the M1 and the P1 domains containing a potential N-glycosylation; a phosphorylation consensus site at the N-terminus; a phosphorylation consensus site at the C-terminus; and a phosphorylation consensus site between the M2 and M3 domains.

As stated above, a claim is adequately described when the specification conveys to one skilled in the art that the inventors had possession of the claimed invention at the time of filing. Possession can be shown by a description of sufficient, relevant identifying characteristics, such as a structural features combined with functional characteristics coupled with a known or disclosed correlation between the function and structure.

Here, the claimed functional derivatives of SEQ ID NO. 2 are described by numerous structural features, including two pore domains and four transmembrane domains. The claimed functional derivatives of SEQ ID NO. 2 are further described by the functional characteristic of being suitable for transporting potassium across a membrane with weak inward rectification properties. This functional characteristic is correlated with at least the two pore domain and four transmembrane domain structures of the presently claimed channel proteins (see the specification at pg. 2, last full paragraph to pg. 3, first paragraph). These and the other claimed structural features, combined with the defined biological activity of potassium transport across a membrane with weak inward rectification, are sufficient to distinguish the claimed functional derivatives of SEQ ID NO. 2 from other transmembrane channel proteins. New claim 28 further defines the two pore domains as SEQ ID NO. 5 and NO. 6, respectively. One skilled in the art, from reading the present specification, would therefore recognize that the inventors had possession of the claimed functional derivatives of SEQ ID NO. 2. Claim 27 and its dependent claim 28 are thus

fully described by the present specification, and the Applicants respectfully request that the 35 U.S.C. § 112, first paragraph rejections be withdrawn.

Claim 27 and its dependent claim 28 are also fully enabled by the present specification. As stated above, a specification is enabling if it teaches one skilled in the art how to make and use the claimed invention without undue experimentation. Statements in the specification regarding the manner of making and using the claimed invention, in terms which correspond in scope to those used in describing and defining the claimed subject matter, are considered to be enabling unless there is reason to doubt their objective truth. To establish a *prima facie* case of non-enablement, therefore, the Examiner must provide scientific evidence or cogent reasoning as to why a patent application does not teach how to make or use the claimed invention.

The Examiner has provided no such cogent reasoning or scientific evidence. The Examiner does state that the Applicants have provided no working examples involving the claimed functional derivatives of SEQ ID NO. 2, nor do the Applicants identify which amino acids would are critical in maintaining the functional characteristics of the claimed proteins.

However, "the specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation." MPEP 2164.02. Here, the specification states on pg. 13, first and second full paragraphs that SEQ ID NO. 2 can be modified to produce functionally equivalent derivatives, as long as the properties and structure of the TWIK-1 channel protein is preserved. Thus, the claimed functionally equivalent derivatives must retain at least those structures defined in claims 27 and 28; namely, two pore domains P1 and P2, wherein said P2 domain comprises a GLG sequence; four transmembrane domains M1 to M4; an amino acid loop between the M1 and the P1 domains containing a potential N-glycosylation; a phosphorylation consensus site at

the N-terminus; a phosphorylation consensus site at the C-terminus; and a phosphorylation consensus site between the M2 and M3 domains.

The retention of such structures in the claimed functionally equivalent derivatives can be determined, for example, by determining the amino acid sequence of the derivative, and comparing that sequence to SEQ ID NO. 2. The retention of TWIK-1 channel functionality in the claimed functionally equivalent derivatives can be determined, for example, by the functional assays disclosed on pgs. 9-12 of the present specification.

The claimed functionally equivalent derivatives must also retain those amino acids which are necessary for retaining the claimed TWIK-1 structures and functionality. One skilled in the art can readily determine which amino acids can and cannot be modified or suppressed by subjecting a putative functionally equivalent derivative to the functional assays outlined on pgs. 9-12 of the present specification.

The sequencing of a putative functionally equivalent derivative of SEQ ID NO. 2 can be accomplished with no more than the application of routine molecular biological or chemical techniques. Thus, such experimentation is not undue. Moreover, as the determination of TWIK-1 functionality for a given protein is described in detail on pgs. 9-12 of the specification, such experimentation is also not undue. The present specification is thus enabling for the claimed functionally equivalent derivatives of SEQ ID NO. 2. Withdrawal of the 35 U.S.C. § 112, first paragraph rejection of claim 27 is therefore respectfully requested.

In view of the foregoing, the Applicants respectfully submit the Application is now in condition for allowance, which is respectfully requested.

Sincerely,

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invited review

Molecular and functional properties of two-pore-domain potassium channels

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Lesage, Florian, and Michel Lazdunski. Molecular and functional properties of two-pore-domain potassium channels. *Am J Physiol Renal Physiol* 279: F793–F801, 2000.—The two-pore-domain K^+ channels, or K_{2P} channels, constitute a novel class of K^+ channel subunits. They have four transmembrane segments and are active as dimers. The tissue distribution of these channels is widespread, and they are found in both excitable and nonexcitable cells. K_{2P} channels produce currents with unusual characteristics. They are quasi-instantaneous and noninactivating, and they are active at all membrane potentials and insensitive to the classic K^+ channel blockers. These properties designate them as background K^+ channels. They are expected to play a major role in setting the resting membrane potential in many cell types. Another salient feature of K_{2P} channels is the diversity of their regulatory mechanisms. The weak inward rectifiers TWIK-1 and TWIK-2 are stimulated by activators of protein kinase C and decreased by internal acidification, the baseline TWIK-related acid-sensitive K^+ (TASK)-1 and TASK-2 channels are sensitive to external pH changes in a narrow range near physiological pH, and the TWIK-related (TREK)-1 and TWIK-related arachidonic acid-stimulated K^+ (TRAAK) channels are the first cloned polyunsaturated fatty acids-activated and mechanogated K^+ channels. The recent demonstration that TASK-1 and TREK-1 channels are activated by inhalational general anesthetics, and that TRAAK is activated by the neuroprotective agent riluzole, indicates that this novel class of K^+ channels is an interesting target for new therapeutic developments.

two-pore-domain channels; mechanosensitivity; anesthetics

POTASSIUM CHANNELS ARE PROTEIN complexes that form K^+-selective pores in biological membranes. They allow the passive transport of K^+ through membranes. They play a major role in the control of K^+ homeostasis and cell volume but also in physiological functions that are associated with modifications of the electrical membrane potential such as neurotransmitters and hormone secretion and neuronal and muscular excitability. A wide variety of K^+ currents have been recorded in vivo that can be distinguished according to their functional and pharmacological properties.

A considerable cloning effort during the last ten years has revealed the structure of many of these channels. They are multimers of hydrophobic subunits that form the ionic pore itself, often associated with accessory subunits (for review, see Refs. 9 and 19). More than 60 pore-forming subunits have now been cloned in mammals. They are classified into three groups according to their membrane topology. The largest group comprises subunits that contain a hydrophobic core with six transmembrane segments (6TMS) and one pore (1P) domain. This domain is directly involved in the formation of the selectivity filter that pro-

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vides the specificity for K^+ transport. The second family is formed by pore-forming subunits having only 2TMS and 1P domain. The extensive characterization of these two types of cloned subunits both in vitro and in vivo, as well as the isolation of associated regulatory subunits, has allowed the reconstitution of many different types of K^+ channels such as voltage-gated K^+ channels, Ca^{2+}-dependent K^+ channels, ATP-sensitive K^+ channels, G protein-coupled K^+ channels, and inward rectifiers.

The last group of K^+-selective pore-forming subunits corresponds to proteins with 4TMS and 2P domains, instead of one as for the other K^+ channel families. This unique feature is at the origin of their name, 2P domain K^+ channels or K_{2P} channels. The discovery of this family is recent. At present, eight different K_{2P} have been cloned in rodents and humans. They can be put in four different classes: TWIK-1 and TWIK-2 (for Tandem of P domains in Weak Inward rectifier K^+ channels) are weak inward rectifiers; TREK-1 (for TWIK-RElated K^+ channel) and TRAAK [for TWIK-Related Arachidonic Acid (AA)-stimulated K^+ channel] are polyunsaturated fatty acids (FA)- and stretch-activated K^+ channels; TASK-1 and TASK-2 (for TWIK-related Acid-Sensitive K^+ channels) are acid-sensitive K^+ channels; and KCNK6 and KCNK7 are silent subunits that probably need a partner to become active. The purpose of this review is to provide the reader with the most complete description of molecular and functional properties of K_{2P} channels.

THE K_{2P} CHANNEL GENE FAMILY

Because of its unique conservation between subunits belonging to the 6TMS/1P and 2TMS/1P classes, the pore domain was used extensively to identify, from public DNA sequence databases, new sequences potentially coding for novel K^+ channel subunits. This approach resulted in the cloning of the 8TMS/2P channel TOK1 from yeast (20, 29, 47, 64) and the human 4TMS/2P channel TWIK-1 (30). Subsequently, seven TWIK-1-related subunits were cloned by degenerated PCR and by computational mining of DNA databases (7, 10, 13, 14, 22, 28, 31, 44, 48, 51) (Table 1). These subunits are 307–499 amino acid residues long and share a common structural organization as shown in Fig. 1*A*. The major structural features are the four potential transmembrane segments (M1–M4), the 2P domains (P1 and P2), short NH_2-terminal and long COOH-terminal cytoplasmic parts, and an extended extracellular loop between M1 and P1. Outside the pore domains, these subunits do not share significant sequence homologies with the 6TMS/1P and 2TMS/1P subunits. Figure 1*B* shows a dendrogram deduced from the sequence alignment of the K_{2P} subunits cloned from mice and humans. The sequence homology between these subunits is usually low (not exceeding 45% for the human subunits), except between TWIK-1 and TWIK-2 (58%) and between TREK-1 and TRAAK (54%). This sequence conservation is associated with a conservation of some of the functional properties. However, this is not always the case. For example, TASK-1 and TASK-2 have similar functional properties (see BASELINE ACID-SENSITIVE TASK-1 AND TASK-2 and Table 2) but are not particularly sequence related (<33% of homology). This indicates that sequence comparison is

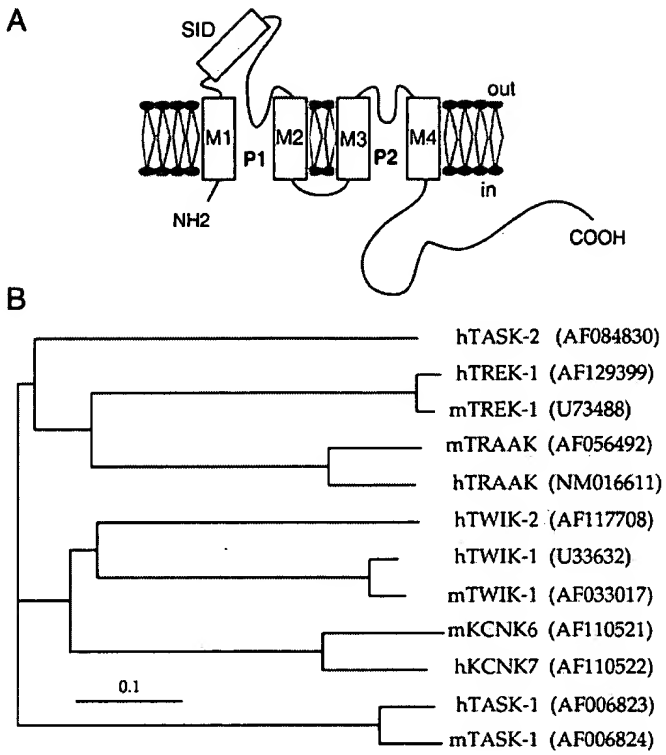

Fig. 1. Membrane topology and diversity of 2-pore (2P)-domain K^+ channel (K_{2P}) subunits. *A*: schematic representation of a K_{2P} subunit. The transmembrane domains (M1–M4) and 2P domains (P1 and P2) are noted. The potential amphipathic α-helix that is involved in the formation of homodimers is noted in the self-interacting domain (SID). *B*: dendrogram of the K_{2P} channels cloned in humans. Seven or eight homologous channels have already been identified, depending on the fact that KCNK6 and KCNK7 may be products of orthologous genes. This dendrogram was established with the use of ClustalW and Treeview software, and the scale bar is in arbitrary units. GenBank accession nos. are indicated in brackets. TWIK-1 and TWIK-2, Tandem of P domains in Weak Inward rectifier K^+ channels; TASK-1 and TASK-2, TWIK-related Acid-Sensitive K^+ channels; TREK-1, TWIK-RElated K^+ channel; TRAAK, TWIK-Related Arachidonic Acid-stimulated K^+ channel; h, human; m, mouse.

Table 1. *Chromosomal locations of K_{2P} channel genes*

Channel	Gene	Chromosomal Location	Reference No.
TWIK-1	*KCNK1*	1q41-42	35
TREK-1	*KCNK2*	1q41	32
TASK-1	*KCNK3*	2p23	32
TRAAK	*KCNK4*	11q13	34
TASK-2	*KCNK5*	6p21	48
TWIK-2	*KCNK6*	19q13	18
KCNK7	*KCNK7*	11q13	51

K_{2P}, 2-pore-domain K^+ channel; TWIK-1 and TWIK-2, Tandem of P domains in Weak Inward rectifier K^+ channels; TASK-1 and TASK-2, TWIK-related Acid-Sensitive K^+ channels; TREK-1, TWIK-Related K^+ channel; TRAAK, TWIK-Related Arachidonic Acid-Stimulated K^+ channel.

Table 2. *Comparison of the functional characteristics of the K_{2P} channels*

		Electrophysiology		Pharmacology			Reference No(s).
		Behavior	Conductance	Blockers	Openers	Regulation	
TWIK-1	336 aa	Inward rectifier	34 pS	Ba^{2+}/quinidine		$[H^+]_i$, PKC activators	30
TWIK-2	313 aa	Inward rectifier	ND			$[H^+]_i$, PKC activators	7
TASK-1	395 aa	GHK rectifier	14 pS	Zn^{2+}/Local anesthetics	General anesthetics	$[H^+]_e$, $pIC_{50} = 7.3$	10, 22, 28, 41
TASK-2	499 aa	GHK rectifier	60 pS	Quinidine Local anesthetics		$[H^+]_e$, $pIC_{50} = 8.3$	48
TREK-1	411 aa	Outward rectifier	100 pS	Quinidine/Gd^{3+}/cationic membrane cup formers	PUFAs/general anesthetics/anionic membrane crenators	PKA and PKC activators, $[Na^+]_e$, $[H^+]_i$, membrane stretch ($P_{0.5} = -36$ mmHg)	13, 38, 41, 42
TRAAK	393 aa	GHK rectifier	45 pS	Gd^{3+}/cationic membrane cup formers	Riluzole/PUFAs/general anesthetics/anionic membrane crenators	Membrane stretch ($P_{0.5} = -46$ mmHg)	14, 37

aa, Amino acid; ND, not determined; PUFA, polyunsaturated fatty acid; GHK, Goldman-Hodgkin-Katz; $[H^+]_i$ and $[H^+]_e$, intracellular and extracellular H^+ concentration, respectively; $P_{0.5}$, pressure to induce half-maximal activation.

not sufficient for predicting the functional properties of K_{2P} channels.

Pore-forming K^+ channel subunits with 4TMS and 2P domains have also been identified in *Drosophila* (17) and *Caenorhabditis elegans* (59). In the nematode, >50 genes may encode K^+ channels belonging to this family. Because a total of 70–80 genes encode for potential pore-forming K^+ channel subunits in this animal model, the K_{2P} channels form the largest class. Sequence homology between nematode K_{2P} subunits, and between them and the human subunits, is low, with usually <35% of amino acid similarity. Except for TASK-1, no K_{2P} channel orthologs can be clearly identified between human and *C. elegans* (33). This is also the case for the K_{2P} channels in *Drosophila*, where three genes are related to TASK-1, whereas the eight others do not seem specially related to any human channel (unpublished observations). Whether a very large family of K_{2P} genes exists in mammals as in *C. elegans* will soon be verified, thanks to the human genome program. By mining the databases corresponding to the already sequenced part of the genome (4 K_{2P} genes in 13% of the genome), one can make the tentative extrapolation that ~30 K_{2P} channel genes will be expressed in humans.

DIMERIZATION OF K_{2P} SUBUNITS

TWIK-1 self-associates to form disulfide-bridged homodimers (36). Such dimers contain 4P domains, which have been previously found to be essential in the formation of the K^+-selective pore in other channels of the 6TMS/1P or 2TMS/1P types, which all form noncovalent tetramers. In mouse brain, the apparent molecular mass of TWIK-1 is 81 kDa when analyzed by Western blot in the absence of a reducing agent, and 40 kDa in the presence of such an agent (31). This assembly involves a 44-amino acid domain located in the M1P1 interdomain that is sufficient to promote the self-dimerization of fusion proteins. Secondary structure analysis of this domain predicts that it forms an amphipathic α-helix with a regular occurrence of charged residues and large apolar residues. This pattern is typical of the interdigitating helices. Cysteine 69, which is part of the self-interacting domain, is implicated in the formation of the interchain disulfide bond. Replacing this cysteine with either a serine residue in TWIK-1 (36) or an alanine residue in TWIK-2 (7, 30, 31) results in the loss of functional expression. Finally, the extracellular location of the M1P1 domain of TWIK-1 was verified both by demonstrating the *N*-linked glycosylation of the asparagine 95 present in the M1P1 domain and by immunodetecting this domain at the surface of unpermeabilized cells (36).

Since the original characterization of TWIK-1, these observations have been extended to the other K_{2P} channels. All cloned subunits except TASK-1 contain a cysteine residue at a position equivalent to cysteine 69 of TWIK-1 and all these subunits except TASK-1 are able to form covalent homodimers when heterologously expressed in insect or COS cells (unpublished observations). The covalent dimerization of TREK-1 and TRAAK was also observed in synaptic membranes. Despite low-sequence conservation between the M1P1 domains of the different K_{2P} channels, the prediction of their secondary structure is always an amphipathic α-helix. This suggests that the ability of this domain to self-interact is a property that is common to all K_{2P} channels. The sequence of the M1P1 extracellular domain in the different K_{2P} channels is very variable. Besides its role in the dimerization, this extracellular domain might well bind regulatory factors or extracellular ligands that would participate to the control of activity of this particular class of channels. However, data supporting such a role are not yet available.

THE WEAK INWARD RECTIFIERS TWIK-1 AND TWIK-2

When expressed in heterologous expression systems, both TWIK-1 and TWIK-2 produce constitutive K^+ currents of weak amplitude (7, 30, 31). These currents are quasi-instantaneous and noninactivating. A saturation of outward currents is observed for high depolarization indicating a weak inward rectification. TWIK-1 has a unitary conductance of 34 pS in symmetrical 140 mM K^+ (30). As expected for a time-independent current active at all potentials, its expression is associated with a setting of the resting potential close to the K^+ equilibrium potential (E_K) (30).

TWIK-1 but not TWIK-2 is blocked by Ba^{2+}, quinine, and quinidine (50 μM > IC_{50} > 100 μM). Both channels are slightly or not sensitive to the classic K^+ channel blockers tetraethylammonium (TEA), 4-aminopyridine (4-AP), and Cs^+. The regulatory properties are similar between the two channels (7, 30). They are regulated in opposite ways by activators of protein kinase C (PKC) and by acidification of the internal medium. PKC activation increases the TWIK currents, whereas acidification inhibits them. For TWIK-1, it has been demonstrated that these effects are indirect (30). The mutation of the unique consensus site for PKC phosphorylation does not modify the sensitivity to agents that activate PKC, and the inhibition by acidification is not seen in the inside-out patch configuration when the internal side of the channel is faced to the acidic medium. TWIK-1 and TWIK-2 are not sensitive to changes in extracellular pH and to treatments that activate protein kinase A (PKA).

The TWIK channels have widespread tissue distribution in adult mice (1, 31) and humans (Fig. 2). They are present in all examined tissues except in skeletal muscle. Together with their functional properties, their wide distribution suggests that these channels could be involved in the control of background K^+ conductances in many cell types. Similar currents have been recorded in pancreatic acinar cells, where they maintain the resting membrane potential (52, 53). Like TWIK-1 and TWIK-2, these currents are inhibited by intracellular acidity, and they are not sensitive to TEA and 4-AP. However, their insensitivity to Ba^{2+} suggests that they are more probably carried by TWIK-2 than TWIK-1 (52). Weak inward rectifiers have also been reported in hepatocytes (56). Moreover, in these cells, intracellular acidification is known to cause a depolarization associated with the inhibition of a quinine-sensitive K^+ conductance (4). Finally, compared with TWIK-2, TWIK-1 is highly expressed in the brain. In situ hybridization (31) indicates a distribution that is restricted to a few regions, and the strongest signals were seen in hippocampus and in cerebellar granule and Purkinje cells. TWIK-1 is expected to have a major role in the maintenance of the resting potential of neuronal cells that express it.

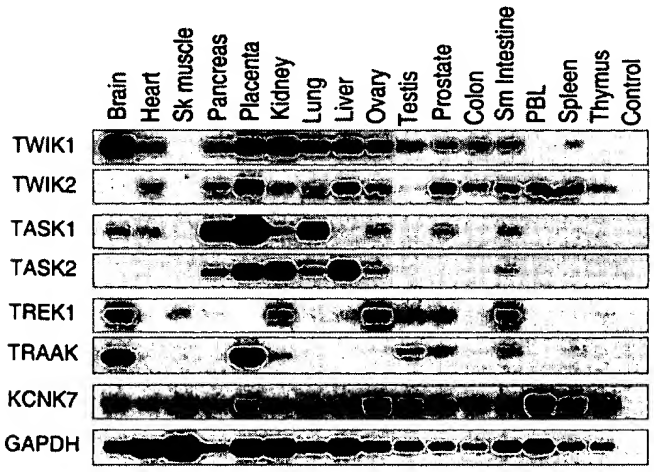

Fig. 2. Distribution of K_{2P} channels in adult human tissue. DNA fragments were PCR amplified by using specific primers and analyzed by Southern blot by using internal ^{32}P-labeled oligonucleotides. PBL, peripheral blood leukocytes; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase.

THE BASELINE ACID-SENSITIVE TASK-1 AND TASK-2 CHANNELS

TASK-1 was the first cloned mammalian K^+ channel to produce currents with all the characteristics of background or baseline conductances (10, 22, 28). These currents are time and voltage independent: they are instantaneous with voltage changes (they do not display activation, inactivation, or deactivation kinetics), and their current-voltage relationships fit the curves predicted from the constant field theory for simple electrodiffusion through an open K^+-selective pore. TASK-1 currents show an outward rectification in physiological asymmetric K^+ conditions that is not observed in symmetric K^+ conditions. The rectification can be approximated by the Goldman-Hodgkin-Katz current equation that predicts a curvature of the current-voltage relationships in asymmetric K^+ conditions. Unlike TASK-1, TASK-2 currents display rapid activation kinetics (48). These kinetics are fitted with a single exponential characterized by time constants of 60 ms at +50 mV. Despite this difference, TASK-2, like TASK-1, shows no rectification other than that predicted by the Goldman-Hodgkin-Katz current equation and lacks intrinsic voltage sensitivity. TASK-1 and TASK-2 currents are highly flickering and have unitary conductances of 14 and 60 pS, respectively, in symmetric 150 mM K^+ (24, 28, 48).

TASK-1 and TASK-2 are relatively insensitive to Ba^{2+}, Cs^+, TEA, and 4-AP. TASK-2 (and to a lesser extent, TASK-1) is blocked by quinine (IC_{50} = 22 μM) and quinidine (65% of inhibition at 100 μM). Zn^+ is a better blocker of TASK-1 (IC_{50} = 175 μM) than of TASK-2 (<15% of inhibition at 100 μM). Both TASK channels are inhibited by the local anesthetics lidocaine and bupivicaine, bupivicaine being the more potent blocker (IC_{50} = 68 μM for TASK-1 and 81% of inhibition of TASK-2 at 1 mM) (25, 28, 48). TASK-1 was recently shown to be opened by volatile general anesthetics, halothane and isoflurane, at concentrations used in human general anesthesia (41).

The essential property of TASK currents is their extreme sensitivity to variations in external pH in a narrow physiological range (10, 24, 28, 48). As much as 90% of the maximal TASK-1 current is recorded at pH 7.7 and only 10% at pH 6.7. The pH value for 50% of inhibition is 7.3 at 0 mV (10). The sensitivity of TASK-2 is less sharp, with 90% of the current at pH 8.8 and 10% at pH 6.5. The pH value for 50% of inhibition is 8.3 at 0 mV (48). In both cases, the inhibition and activation produced no modification of current kinetics. The pH effects are due to a variation in the number of active channels and not in the single-channel conductances (48). TASK channels are insensitive to application of PKC activators, and only TASK-1 has been shown to be decreased by application of PKA activators (28).

The distribution of TASK channels is more restricted than the TWIK expression pattern (Fig. 2). However, TASK-1 and TASK-2 are present in many different tissues (pancreas, placenta, kidney, lung, liver, ovary, prostate, and small intestine), where they are supposed to contribute to maintenance of resting membrane potentials and/or to K^+ transport associated with recycling or secretion. Both channels are present in nonexcitable tissues, but only TASK-1 is present in brain and heart. In rodent heart, TASK-1 is mainly expressed in atrial myocytes. In the brain, its expression is neuronal. The literature on baseline or leak K^+ channels is not abundant compared with that on other types of K^+ channels. This probably originates from the fact that they are difficult to study. They are voltage and time independent, and they have no specific pharmacology. Background K^+ channels have been recorded in *Bufo* smooth muscle cells (39), in rat ventricular myocytes (2) and carotid bodies (5), and in bovine adrenocortical cells (12). They have also been recorded in different preparations of neuronal cells, in invertebrates, in *Aplysia* sensory neurons (55), leech AP neurons (43), *Lymneae* neurons (15), and lobster stretch receptor neurons (57), as well as in vertebrates, in bullfrog sympathetic ganglia (27), *Xenopus* myelinated nerve (26) and demyelinated axons (61), guinea pig submucosal neurons (54), and rat hippocampal (23, 45, 46) and premotor respiratory neurons (58). All these channels are quasi-instantaneous and noninactivating. They are also not gated by potential and exhibit outward rectification in physiological K^+ conditions. When determined, their single-channel behavior is flickering. The majority of these currents are insensitive to TEA and 4-AP, and Ba^{2+} differentially affects them. TASK-1 could be a major contributor to these background conductances in excitable cells. In addition to maintenance of resting potential, it could also play a role in the modulation of electrical activity of these cells. The modulation of TASK-1 by external protons probably has important implications for its physiological function. Stimulus-elicited pH shifts have been characterized in a variety of neural tissues by using extracellular pH-sensitive electrodes. Electrical stimulation of Schaeffer collateral fibers in the hippocampal slice, or light stimulation of the retina or parallel fibers in cerebellum, produces pH shifts corresponding to bursts of H^+ or OH^-, creating small pH variations from the external physiological pH value of 7.4 (up to 0.3 pH unit in the alkaline or acidic direction). The variations might actually be larger in range or shorter in time course in the vicinity of the synaptic cleft. TASK-1 contains a potential site of interaction with synaptic proteins containing PDZ-domains, suggesting that it could be located at synapses. The strong modulation of TASK-1 by external pH favors the idea that extracellular variations in H^+ concentrations can be a modulator of neuronal activity.

THE UNSATURATED FA- AND STRETCH-ACTIVATED TREK-1 AND TRAAK CHANNELS

TREK-1 and TRAAK have unique functional properties and represent the first cloned polyunsaturated FA and stretch-activated K^+ channels. Like TASK-1, these channels produce instantaneous currents, which are outwardly rectifying in physiological K^+ gradient. In high symmetric K^+, TRAAK currents are linear like those of TASK-1, but TREK-1 still presents an outward rectification for strong hyperpolarizations. TREK and TRAAK channels are highly flickering, and their unitary conductances are 100 and 45 pS, respectively, in symmetric 150 mM K^+ (13, 14).

In heterologous expression systems, TREK-1 and TRAAK currents have a low basal activity compared with the TASK channels. They can be strongly activated by application of AA (14, 34, 37, 42). This activation is reversible and concentration dependent. It is not prevented when the AA perfusion is supplemented with a mixture of inhibitors of the AA metabolism pathway, supporting the idea that the AA effect is direct and not due to another eicosanoid. This effect is specific to unsaturated FAs. Oleate, linoleate, arachidonate, eicosapentaenoate, and docosahexaenoate all strongly activate TREK-1 and TRAAK, whereas saturated FAs such as palmitate, stearate, and arachidate are ineffective. Another effective way for activating these channels is the application of stretch to the cell membrane. Both channels are activated by shear stress, cell swelling, and negative pressure (37, 38, 42). The pressure to induce half-maximal activation is -36 mmHg for TREK-1 and -46 mmHg for TRAAK. Disruption of the cytoskeleton by either biological or mechanical means (colchicine, cytochalasin, or membrane excision) potentiates the opening by membrane stretch. This result suggests that these channels are tonically repressed by the cytoskeleton but that their mechanogating does not require the integrity of the cytoskeleton. This also implies that the activating force is coming directly from the bilayer membrane. Moreover, agents that insert preferentially in one of the leaflets of the membrane and that modify the cell shape cause modification of the activity of these channels. The lipid bilayer anionic or neutral crenators open the channels, whereas the cationic cup formers inhibit both basal and stimulated activities (37, 42).

As expected for stretch-activated channels, TREK-1 and TRAAK are reversibly blocked by micromolar concentrations of Gd^{3+}. They are resistant to TEA and 4-AP and slightly sensitive to Ba^{2+} at high concentrations. TREK-1 is blocked by quinidine (IC_{50} = 100 μM). Both channels are activated by riluzole, a neuroprotective agent used in the treatment of amyotrophic lateral sclerosis (11).

Compared with TRAAK, TREK-1 has additional features. Inhalational general anesthetics halothane and isoflurane activate TREK-1 as well as TASK-1 (41). However, unlike TASK-1, TREK-1 is also activated by chloroform and diethyl ether (41). In terms of regulation, TREK-1 but not TRAAK is inhibited by activators of PKC and PKA (13, 14). The phosphorylation site by PKA has been localized to the cytoplasmic COOH part of the channel (serine 333) (42). Finally, TREK-1 is opened by internal acidification. Lowering intracellular pH shifts the pressure-activation relationships toward positive values and leads to channel opening at atmospheric pressure (38). By mutagenesis, it has been shown that the COOH terminus of TREK-1 is critically involved in mechanogating, AA activation, and intracellular pH sensitivity (38, 42).

Human TREK-1 is mainly expressed in brain, ovary, and small intestine (Fig. 2). TRAAK is highly expressed in brain and placenta (34) (Fig. 2). This distribution is not strictly identical in the mouse, where TRAAK is specifically expressed in neuronal cells, whereas TREK-1 is present in more tissues than in humans (13, 14). Both channels are found in hippocampus, neocortex, cerebellum, brain stem nuclei, and olfactory bulb (13, 14). However, immunolocalization by specific antibodies has shown that the two channels have different subcellular locations. TRAAK is mainly present in soma and, to a lesser degree, in axons and dendrites (49), whereas TREK-1 is concentrated in dendrites in almost all neuronal types expressing the channel (unpublished observations).

TREK-1 shares many of the biophysical and pharmacological properties of the *Aplysia* S-type channel (42). This channel is expressed in sensory neurons of the mollusk, where it is known to play a major role in the regulation of synaptic transmission in *Aplysia* synapses (6, 55). Both channels are outwardly rectifying, time independent, and resistant to Ba^{2+}, TEA, and 4-AP, and they are opened by volatile general anesthetics (41, 60). AA and membrane stretch activate both channels, and they are blocked by serotonin via the PKA-cAMP pathway. The closure of the *Aplysia* channel by cAMP causes slow depolarization and a broadening of action potentials in the cell body. The enhanced excitability results in an augmentation of neurotransmitter release from sensory neurons. On the other hand, the opening of the channel by AA causes reduced excitability and lowering of neurotransmitter release. Background K^+ channels with properties similar to TREK currents were also recorded from mammalian neurons that are activated by application of baclofen, which binds to the $GABA_B$ receptor that is negatively coupled to adenylate cyclase (45, 58), or that are activated by application of volatile anesthetics (60). Background K^+ channels activated by AA have been described in mammalian neurons (23, 46) and in heart (21) and smooth muscle cells (39). Our results suggest that TREK-1 and/or TRAAK underlies some of these currents. They also suggest that the distribution of this particular class of K^+ channels in the central nervous system is much more widespread than previously believed.

THE SILENT SUBUNITS KCNK6 AND KCNK7

The KCNK6 subunit has been cloned from mouse (51). It has the classic 4TMS/2P topology and contains a Ca^{2+}-binding EF hand motif. Although KCNK6 is able to dimerize as other functional K_{2P} subunits when heterogously expressed in COS cells, it remains in the endoplasmic reticulum and is unable to generate ion channel activity at the cell surface. Mutagenesis experiments suggest that KCNK6 is not an intracellular channel but rather a subunit that needs to associate with a yet undiscovered partner to reach the plasma membrane (51). KCNK6 is mainly expressed in the embryo and in adult tissues such as eye, lung, and stomach. The highest level of expression is found in the eye, where in situ hybridization and immunohistochemistry showed that KCNK6 is only expressed in ganglion cells and in some neurons of the inner nuclear layer. In the mammalian retina, the first spontaneous Ca^{2+} waves are observed at postnatal *day 2* and are thought to result from Ca^{2+} influx associated with a burst of action potentials seen in ganglion at this developmental stage. The early appearance of the KCNK6 in development, the fact that it has a Ca^{2+}-binding site potentially conferring Ca^{2+} sensor properties, and its selective expression in ganglion cells suggest that this channel could play a role in the modulation of the electrical signal in the retina.

A human subunit, KCNK7, closely related to KCNK6, has been cloned (51). Despite 94% of sequence homology, KCNK7 and KCNK6 display several differences that question the possibility that these subunits are the products of orthologous genes in humans and mice. KCNK7 does not contain the EF hand motif of KCNK6, and its tissue distribution is wider than that of KCNK6, with the highest level of expression in peripheral blood leukocytes. In addition, a unique feature of KCNK7 is the presence of an unusual sequence in its second pore domain. An important element of the signature of K^+ channel function has long been recognized as being the pore domain GYG sequence. In the K_{2P} channels, this GYG motif is replaced by GFG (TASK-1, TASK-2, TREK-1, and TRAAK) or GLG (TWIK-1 and TWIK-2). In KCNK7, a glutamic residue (GLE) is found instead of the strictly conserved glycine residue (GLG). This unusual sequence could be associated with a change in ionic selectivity. However, KCNK7, like KCNK6, failed to express channel activity by itself.

K_{2P} CHANNELS IN THE KIDNEY

In the kidney, K^+ channels are involved in the control of negative membrane potential, the regulation of cell volume, and K^+ recycling or secretion. Several K^+ conductances have been recorded in this tissue, with specific properties and locations in distinct cell types and membrane domains (for review, see Ref. 16). However, the corresponding molecular structures are not always clearly established. An important exception is ROMK, a 2TMS/1P channel that shares biophysical properties with a channel of cortical collecting ducts (CCDs) that constitutes the main way of K^+ secretion in the principal cells. This secretion is essential for permitting sustained Na-K-2Cl cotransporter activity and renal Na^+ reabsorption. Mutations in the ROMK gene cause Bartter's syndrome in humans, which is associated with salt wasting and hypokalemic alkalosis. Another small-conductance 2TMS/1P channel and two additional 6TMS/1P channels, cGMP gated (63) or glibenclamide sensitive (62), have been found in the kidney, but their exact roles are not completely understood.

Figure 2 shows that all K_{2P} subunits are expressed in human kidney, the most abundant being TWIK-1 and TASK-2. TWIK-1 is also highly expressed in rat kidney, where it was immunolocalized (8). TWIK-1 is present in the brush-border membrane of the proximal convoluted tubules, in the thick ascending limb of the loop of Henle, and in the collecting duct intercalated cells, with intracellular and apical localization. Another study has shown by RT-PCR that TWIK-1 is localized in the distal nephron in rabbit kidney (40). K^+ channels with a relatively low conductance have been described in the apical membrane of CCD cells. In addition to the conductance (30–35 pS), they share with TWIK-1 a low sensitivity to TEA and an inhibition by internal acidosis. However, the sensitivity to PKC effectors and cAMP via PKA is different between TWIK-1 and these particular channels. Whether these differences are real or reflect a different cellular context of expression (native cells vs. oocytes) remains unknown. Nevertheless, the particular localization of TWIK-1 suggests that it could play a role in K^+ secretion complementary to ROMK. TASK-2 has been localized by in situ hybridization in human kidney (48). It is present in cortical distal tubules and CCDs. The biophysical and pharmacological properties of TASK-2 do not fit those of the native K^+ channels that have been identified there. A possibility would be that TASK-2 has not yet been recorded in kidney cells, which would not be surprising because of the difficulty in identifying this channel in the absence of a specific pharmacology. Another possibility would be that TASK-2 associates with yet unidentified pore-forming subunits or regulatory proteins to produce active channels in native cells with properties different from those of the cloned channel. As for TWIK-1, the high level of expression of TASK-2 suggests that it plays a significant, and maybe even an important, role in renal K^+ transport.

The stretch-activated TREK-1 channel is also expressed in the kidney. Stretch-activated K^+ currents have been recorded in tubules cells, and it has been proposed that these currents are important in regulating cell volume (50).

CONCLUSION

Background K^+ channels have originally been described in myelinated nerve, where sequential application of TEA, 4-AP, and Cs^+ removed different K^+ conductances. However, after these treatments, axons still exhibited a pronounced outward rectification. A residual K^+ background conductance that was outwardly rectifying, as expected from the constant field theory, was present in nerve to set the resting potential (3). TASK-1 is the perfect background or baseline K^+ channel: it is time and voltage independent, constitutively active, and insensitive to TEA, 4-AP, and Cs^+. In terms of rectification, kinetics of activation, or basal activity, the other cloned K_{2P} channels are not perfect background channels. However, they are very close to this "ideal" behavior and, as expected, are able to polarize the membrane potential. If the cloning of K_{2P} channels has provided access to a class of background K^+ channels, it has also provided access to the molecular characterization of the previously recognized functional class of K^+ channels activated by FAs and stretch. Because of their functional diversity and their widespread distribution, K_{2P} channels are expected to fulfill many physiological roles in addition to setting resting membrane potential. The elucidation of these roles will require finding a specific pharmacology for these channels to better analyze their roles in vivo. The identification of specific blockers and openers is also promising in terms of therapy. Until now, the only widely prescribed class of K^+ channel drugs in clinical use was active on ATP-sensitive K^+ channels. With the recent demonstration that TREK-1 and TASK-1 are activated by volatile general anesthetics, and TRAAK by the neuroprotective agent riluzole, K_{2P} channels now appear as valuable targets for the rational development of new drugs.

NOTE ADDED IN PROOF

Two novel K_{2P} channels, TASK-3 and TREK-2, have been cloned that are structurally and functionally related to TASK-1 and TREK-2, respectively (Kim Y et al. *J Biol Chem* 275: 9340–9347, 2000; Rajan S et al. *J Biol Chem* 275: 16650–16657, 2000; Lesage F et al. *J Biol Chem*. In press.). Recent studies have shown that TASK-1 is important for the control of motoneuron and cerebellar granule cell excitability (Talley EM et al. *Neuron* 25: 399–410, 2000; Millar JA et al. *Proc Natl Acad Sci USA* 28: 3614–3618, 2000); for oxygen sensing in the carotid bodies [Buckler KJ et al. *J Physiol (Lond)* 15: 135–142, 2000]; and for the generation of a high resting membrane potential in adrenal glomerulosa cells (Czirjak G et al. *Mol Endocrinol* 14: 863–8764, 2000). In all these cases, TASK-1 is active at rest, and its closure by neurotransmitter, hypoxia, or hormone is associated with a depolarization of the cell membrane and increase in cell excitability. In addition, another recent study shows that rat TWIK-2 generates inactivating currents of large amplitude, suggesting a particular

role for this channel type in cell electrogenesis (Patel AJ et al. *J Biol Chem*. In press.).

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Potassium Channels: Molecular Defects, Diseases, and Therapeutic Opportunities

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Abstract——**Potassium channels play important roles in vital cellular signaling processes in both excitable and nonexcitable cells. Over 50 human genes encoding various K^+ channels have been cloned during the past decade, and precise biophysical properties, subunit stoichiometry, channel assembly, and modulation by second messenger and ligands have been elucidated to a large extent. Recent advances in genetic linkage analysis have greatly facilitated the identification of many disease-producing loci, and naturally occurring mutations in various K^+ channels have been identified in diseases such as long-QT syndromes, episodic ataxia/myokymia, familial convulsions, hearing and vestibular diseases, Bartter's syndrome, and familial persistent hyperinsulinemic hypoglycemia of infancy. In addition, changes in K^+ channel function have been associated with cardiac hypertrophy and failure, apoptosis and oncogenesis, and various neurodegenerative and neuromuscular disorders. This review aims to 1) provide an understanding of K^+ channel function at the molecular level in the context of disease processes and 2) discuss the progress, hurdles, challenges, and opportunities in the exploitation of K^+ channels as therapeutic targets by pharmacological and emerging genetic approaches.**

I. Background

Potassium channels are a diverse and ubiquitous family of membrane proteins present in both excitable and nonexcitable cells. Members of this channel family play critical roles in cellular signaling processes regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume regulation. Over 50 human genes encoding various K^+ channels have been cloned during the past decade (Fig. 1), and precise biophysical properties, subunit stoichiometry, channel assembly and modulation by second messenger and ligands have been addressed to a large extent. More recently, the crystal structure of a K^+ channel from *Streptomyces lividans* has become available (Doyle et al., 1998).

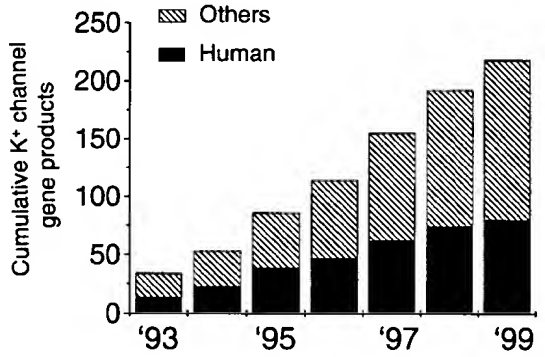

FIG. 1. Growth of genes encoding diverse K^+ channels. The *Shaker* K^+ channel gene was first cloned from *Drosophila* (Papazian et al., 1987). The gene products indicated along the *y*-axis include both K^+ channel α- and auxiliary subunits. The data were obtained from the *Entrez* database of the National Center for Biotechnology Information (NCBI).

Concurrent with this remarkable progress in our understanding of molecular diversity, structure, and function, a growing number of discoveries have linked K^+ channel gene mutations with various diseases. Such diseases of the heart, kidney, pancreas, and central nervous system involve either mutation(s) in K^+ channel gene(s) and/or altered regulation of K^+ channel function. The enhanced understanding of these diseases, facilitated by a combination of genomic and biophysical approaches, has helped our understanding of how various mutations affect channel function, contributes to disease etiology, and rationalizes novel treatment strategies. In this review, we provide a comprehensive overview of our recent understanding of molecular defects of K^+ channels in various diseases and its implications for the development of novel prophylactic or therapeutic approaches targeting distinct types of K^+ channels.

A brief overview of the structural and functional diversity of K^+ channels is initially provided to enable

[2] Abbreviations: Kv, voltage-gated K^+ channel; Aβ, β-amyloid; β-APP, β-amyloid protein precursor; BFNC, benign familial neonatal convulsion; BK_{Ca}, large conductance Ca^{2+}-activated K^+ channel; EA, episodic ataxia; EAG, *ether-a-go-go* K^+ channel; 1-EBIO, 1-ethyl-2-benzimidazolinone; hERG, human *ether-a-go-go*-related K^+ channel; IK_{Ca}, intermediate conductance Ca^{2+}-activated K^+ channel; IKr, cardiac rapid delayed rectifier; IKs, cardiac slow delayed rectifier; IK_{ur}, ultrarapid delayed rectifier; I_{TO}, transient outward delayed rectifier; K_{ATP}, ATP-sensitive K^+; KCsA, K^+ channel from *Streptomyces lividans*; Kir, inward rectifier K^+ channel; KCO, K^+ channel opener; LQT, long-QT syndrome; M-channel, muscarine-sensitive K^+ channel; MiRP, minK related peptide; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; P-loop, pore loop; PS, presenilin; sAPP, secreted form of β-amyloid precursor protein; SK_{Ca}, small conductance Ca^{2+}-activated K^+ channel; SUR, sulfonylurea receptor; TEA, tetraethylammonium; TM, transmembrane segment; TREK, two-pore weak inward rectifier-related K^+ channel.

familiarity with the nomenclature and biophysical and pharmacological characteristics of diverse K^+ channels. Several extensive reviews are already available on this subject that may be consulted for additional details (Doupnik et al., 1995; Coetzee et al., 1999). Diseases involving other voltage-gated ion channels have been reviewed elsewhere (Ackerman and Clapham, 1997; Lehmann-Horn and Rüdel, 1997; Cooper and Jan, 1999).

A. Channel Diversity and Classification

K^+ channels are membrane-spanning proteins that selectively conduct K^+ ions across the cell membrane along its electrochemical gradient at a rate of 10^6 to 10^8 ions/s. To accomplish this, K^+ channels are endowed with a set of salient features: 1) a water-filled permeation pathway (pore) that allows K^+ ions to flow across the cell membrane; 2) a selectivity filter that specifies K^+ as permeant ion species; and 3) a gating mechanism that serves to switch between open and closed channel conformations (Hille, 1992). Since the first gene encoding a K^+ channel was cloned from *Drosophila Shaker* mutant (Papazian et al., 1987), more than 200 genes encoding a variety of K^+ channels have been identified (Fig. 1), all containing a homologous pore segment (S5-S6 linker) selective for K^+ ions (Hartmann et al., 1991; Yellen et al., 1991). Accordingly, a general classification of K^+ channels into families is based upon the primary amino acid sequence of the pore-containing subunit. Three groups with six, four, or two putative transmembrane segments are recognized. These include 1) voltage-gated K^+ channels (*Shaker*-like) containing six transmembrane regions (S1-S6) with a single pore; 2) inward rectifier K^+ channels containing only two transmembrane regions and a single pore; and 3) two-pore K^+ channels containing four transmembranes with two pore regions (Fig. 2). Table 1 lists a generalized classification of various cloned K^+ channel subunits.

1. Six Transmembrane One-Pore Channels. Voltage-gated K^+ channels (Kv), whose members include *Shaker*-related channels, human *ether-a-go-go*-related K^+ channels (hERG), Ca^{2+}-activated K^+ channels, and KCNQ channels, are activated by depolarization.

a. Pore and Selectivity Filter. The tripeptide sequence motif G(Y/F)G located in the S5-S6 linker is common to the pore or P-loop of these and other K^+ channels and hence is considered as the K^+-selectivity signature motif (Heginbotham et al., 1994). The residues immediately adjacent to either side of this motif are also generally conserved within the K^+ channel superfamily. Four of the pore loop domains contribute to the formation of a functional K^+-conducting pore (MacKinnon, 1991). Accordingly, the heteromultimeric complex of voltage-gated K^+ channels is thought to be composed of four pore loop-containing α-subunits arranged in a tetrameric fashion (MacKinnon, 1995; Jan and Jan, 1997). The external entry to the channel pore consisting of portions of the P-loop and adjacent residues in both S5

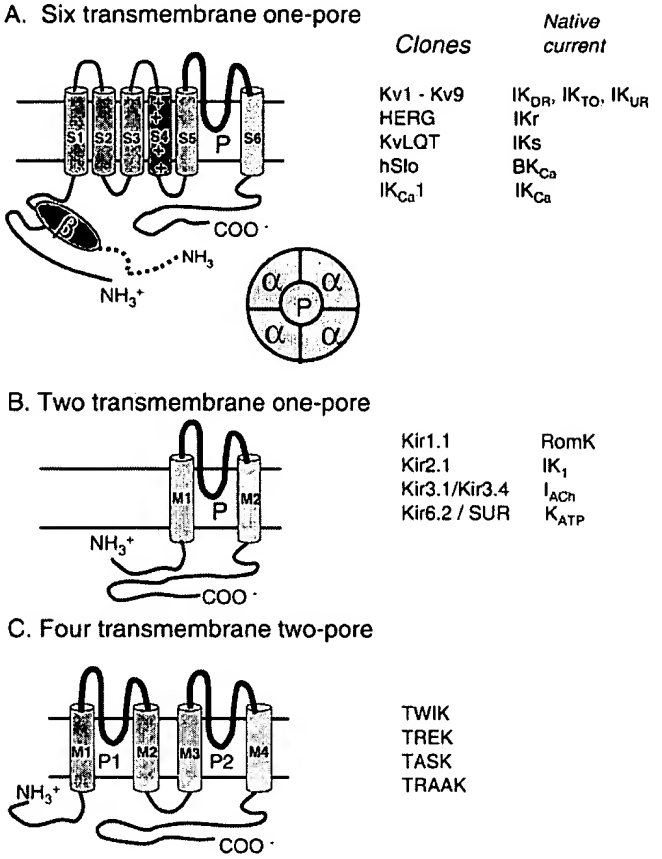

FIG. 2. Schematic representation of the structural classification of K^+ channel subunits. A, 6-TM subunits. The voltage-gated K^+ channels are composed of four subunits each containing six transmembrane segments (S1-S6) and a conducting pore (P) between S5 and S6 with a voltage sensor (positive charge of amino acid residues) located at S4. Some of the voltage-gated K^+ channels include an auxiliary β-subunit (Kvβ), which is a cytoplasmic protein with binding site located at the N terminus of the α-subunit. The inset shows the general assembly of K^+ channels. The homotetrameric K^+ channel consists of four identical subunits while different α-subunits form heterotetrameric K^+ channels. B, 2-TM subunits. The inward rectifier K^+ channel belongs to a superfamily of channels with four subunits each containing two transmembrane segments (M1 and M2) with a P-loop in between. C, 4-TM subunits. This represents a class of the K^+ channels that has four transmembranes with two P-loops. I_{ACh}, muscarine-activated K^+ current; IK_{DR}, delayed rectifying K^+ current; IK_{TO}, transient outward delayed rectifier; IK_{UR}, ultrarapid delayed rectifier; IKr, cardiac rapid delayed rectifier; IKs, cardiac slow delayed rectifier; IK_1, inward rectifier; TWIK, two-pore weak inward rectifier; TASK, TWIK-related acid-sensitive K^+ channel; TRAAK, TWIK-related arachidonic acid-stimulated K^+ channel.

and S6 segments constitutes binding sites for toxins and K^+ channel blockers (MacKinnon and Miller, 1988; MacKinnon et al., 1990; Yellen et al., 1991; Goldstein et al., 1993; Pascual et al., 1995). On the other hand, the internal vestibule of pore composed of residues from S5 and S6 segments facing the intracellular side contributes to binding sites for compounds such as 4-aminopyridine, tetraethylammonium, and quinidine (Choi et al., 1993; Lopez et al., 1994; Shieh and Kirsch, 1994; Yeola et al., 1996). The S4-S5 linker lies close to the permeation pathway and forms part of the receptor for the inactivation ball (Isacoff et al., 1991).

b. Voltage Sensor and Channel Activation. In voltage-dependent ion channels, membrane depolarization

TABLE 1
Potassium Channel Genes and Ancillary Subunits: Localization, Modulators, and Diseases

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms	References
Voltage-gated K^+ channels (*Shaker*)	*KCNA1*	Kv1.1	12p13	Neurons, heart, retina, pancreatic islet	Blocker: α-DTX, HgTX1, MgTX	Episodic ataxia/myokymia syndrome	Ramashwami et al., 1990 Litt et al., 1994 Browne et al., 1994, 1995
						Missense mutations (11 variants)	Adelman et al., 1995 Albrecht et al., 1995 Comu et al., 1996 Boland et al., 1999 D'Adamo et al., 1999
	KCNA2	Kv1.2	1	Brain, heart, pancreatic islet	Blocker: CTX, α-DTX, HgTX1, MgTX, NxTX		Ramashwami et al., 1990 Klocke et al., 1993
	KCNA3	Kv1.3	1p21-p13.3	Lymphocyte, brain, lung, thymus, spleen	Blocker: AgTX2, α-DTX, HgTX1, KTX, MgTX, CTX, NxTX, UK78282, WIN 17317-3, correolide		Grissmer et al., 1990 Attali et al., 1992 Folander et al., 1994 Hanson et al., 1999
	KCNA4	Kv1.4	11q13.4-q14.1	Brain, heart, pancreatic islet	Blocker: UK78282		Tamkun et al., 1991 Philipson et al., 1993 Hanson et al., 1999
	KCNA5	Kv1.5	12p13	Brain, heart, kidney, lung, skeletal muscle	Blocker: 4-AP, clofilium, loratadine, perhexiline		Curran et al., 1992 Phromchotikul et al., 1993 Albrecht et al., 1995
	KCNA6	Kv1.6	12p13	Brain	Blocker: α-DTX		Grupe et al., 1990 Klocke et al., 1993 Albrecht et al., 1995
	KCNA7	Kv1.7	19q13.3	Heart, pancreatic islet, skeletal muscle	Blocker: 4-AP, capsaicin, tedisamil, NxTX, MgTX		Kalman et al., 1998
Voltage- and cGMP-gated K^+ channel	*KCNA10*	Kv1.10	1p13.1	Aorta, brain, kidney	a. Blocker: 4-AP, CTX, ketoconazole, pimozide b. Opener: cGMP		Yao et al., 1995 Orias et al., 1997a Lang et al., 2000
β-subunits for Kv channels	*KCNAB1*	Kvβ1	3q26.1	Brain (Kvβ1.1) Heart (Kvβ1.2)			Rettig et al., 1994 England et al., 1995 McCormack et al., 1995 Majumder et al., 1995 Leicher et al., 1996 Schultz et al., 1996
	KCNAB2	Kvβ2	1p36.3	Brain, heart			Scott et al., 1994 McCormack et al., 1995 Morales et al., 1995 Schultz et al., 1996
	KCNAB3	Kvβ3	17p13.1	Brain			Leicher et al., 1998
Shab	*KCNB1*	Kv2.1-Kv2.2	20q13.2	Brain, heart, kidney, skeletal muscle, retina	Blocker: hanatoxin, TEA		Frech et al., 1989 Hwang et al., 1992 Melis et al., 1995
Shaw	*KCNC1*	Kv3.1	11p15	Brain, muscle, lymphocyte	Blocker: 4-AP		Yokoyama et al., 1989 Grissmer et al., 1992
	KCNC2	Kv3.2	19q13.3-q13.4	Brain	Blocker: 4-AP		Yokoyama et al., 1989 Ito et al., 1992 Haas et al., 1993
	KCNC3	Kv3.3	19q13.3-q13.4	Brain, liver	Blocker: 4-AP		Ghanshani et al., 1992 Ito et al., 1992 Haas et al., 1993
	KCNC4	Kv3.4	1p21	Brain, skeletal muscle	Blocker: 4-AP		Ghanshani et al., 1992 Klocke et al., 1993
Shal	*KCND1*	Kv4.1	Xp11.23-p11.3	Heart, brain, liver, kidney, lung, placenta, pancreas	Blocker: 4-AP		Serodio et al., 1994 Isbrandt et al., 2000
	KCND2	Kv4.2	7q31-32	Brain	Blocker: 4-AP, PaTX		Diochot et al., 1999 Zhu et al., 1999b Postma et al., 2000
	KCND3	Kv4.3	1p13.2	Heart, brain	Blocker: 4-AP, PaTX		Kong et al., 1998 Diochot et al., 1999 Postma et al., 2000
		Kv5.1		Brain			Drewe et al., 1992
		Kv6.1		Brain			Drewe et al., 1992

TABLE 1
Continued

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms	References
	KCNF2	Kv6.2	18q22-18q23	Heart			Zhu et al., 1999a
		Kv8.1	8q22.3-8q24.1	Brain			Hugnot et al., 1996
	KCNS1	Kv9.1		Brain			Salinas et al., 1997
	KCNS2	Kv9.2	8q22	Brain			Salinas et al., 1997
	KCNS3	Kv9.3	2p24	Lung, brain, artery			Patel et al., 1997
	KCNF1	KH1	2p25	Heart, skeletal muscle			Su et al., 1997
	KCNG1	KH2	20q13	Brain, placenta, skeletal muscle			Su et al., 1997
Ether-a-go-go	*KCNH1*	EAG	1q32-q41	Brain			Warmke and Ganetzky, 1994 Occhiodoro et al., 1998
Human *ether-a-go-go*	*KCNH2*	hERG	7q35-q36	Brain, heart	Blocker: clofilium, dofetilide, E4031, LY97241, terfenadine, sertindole	LQT2 a. Missense mutations (7 variants) b. Deletion (2 variants) c. Substitution in intron 3	Jiang et al., 1994 Warmke and Ganetzky, 1994 Curran et al., 1995 Benson et al., 1996 Satler et al., 1996 Itoh et al., 1998 Lee-Chen et al., 1999 Jongbloed et al., 1999 Larsen et al., 2000
	KCNH3	BEC1	12q13	Brain			Banfi et al., 1996 Miyake et al., 1999
	KCNH4	BEC2		Brain			Miyake et al., 1999
MinK	*KCNE1*	MinK	21q22.1-q22.2	Kidney, uterus, heart, cochlea, retina		LQT5 Missense mutations (5 variants)	Murai et al., 1989 Chevillard et al., 1993 Schulze-Bahr et al., 1997 Splawski et al., 1997b Duggal et al., 1998 Tyson et al., 1997
	KCNE2	MinK-related peptide I (MiRP1)	21q22.1			LQT6 Missense mutations (3 variants)	Abbott et al., 1999
	KCNE3	MiRP2		Small intestine, colon, kidney			Schroeder et al., 2000b
KvLQT1	*KCNQ1*	KvLQT1	11p15.5	Heart, cochlea, kidney, lung, placenta, colon	a. Blocker: chromanol-293B b. Opener: L-364,373, stilbenes, fenamates	LQT1; Jervell-Lange Neilsen syndrome a. Missense mutations (17 variants) b. Deletions (6 variants) c. Insertions (2 variants) d. Insertion/deletion (1 variant) e. Splice variant (1)	Russell et al., 1996 Wang et al., 1996 Donger et al., 1997 Neyroud et al., 1997 Splawski et al., 1997a Yang et al., 1997 Ackerman et al., 1998 Kanters et al., 1998 Li et al., 1998 Neyroud et al., 1998 Priori et al., 1998 Splawski et al., 1998 Ackerman et al., 1999 Chen et al., 1999c Jongbloed et al., 1999 Larsen et al., 1999 Murray et al., 1999 Neyroud et al., 1999
	KCNQ2	KvLQT2	20q13.3	Brain, neuron	a. Blocker: TEA, linopirdine, XE991, L-735,821 b. Opener: retigabine	Benign neonatal epilepsy a. Missense mutation (2 variants) b. Insertion (1 variant) c. Deletion (1 variant)	Biervert et al., 1998 Singh et al., 1998 Tinel et al., 1998 Yang et al., 1998 Biervert and Steinlein, 1999 Cooper et al., 2000
	KCNQ3	KvLQT3	8q24	Brain, neuron	a. Blocker: TEA, linopirdine, XE991 b. Opener: retigabine	Benign neonatal epilepsy Missense mutation (2 variants)	Charlier et al., 1998 Hirose et al., 2000

TABLE 1
Continued

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms	References
	KCNQ4	KvLQT4	1p34	Outer hair cells, inner ear, central auditory pathway		Hearing loss a. Missense mutation (6 variants) b. Deletion (1 variant)	Coucke et al., 1999 Kubisch et al., 1999 Talebizadeh et al., 1999 Van Hauwe et al., 2000
	KCNQ5	KvLQT5	6q14	Brain, skeletal muscle	Blocker: linopirdine		Lerche et al., 2000 Schroeder et al., 2000a
Inward rectifier	*KCNJ1*	Kir1.1-Kir1.3	11q24	Kidney, pancreatic islets	Blocker: Ba^{2+}	Bartter's syndrome, type 2; Bartter's syndrome, antenatal onset a. Missense mutation (9 variants) b. Deletion (2 variants)	Ho et al., 1993 Shuck et al., 1994 Yano et al., 1994 Krishnan et al., 1995 Simon et al., 1996b Feldmann et al., 1998
	KCNJ2	Kir2.1		Heart, brain, smooth muscle, skeletal muscle, lung, placenta, kidney	Blocker: Ba^{2+}, spermine, spermidine, Mg^{2+}		Kubo et al., 1993a Raab-Graham et al., 1994
	KCNJ3	Kir3.1	2q24.1	Heart, cerebellum	Blocker: Ba^{2+}		Kubo et al., 1993b Stoffel et al., 1994 Schoots et al., 1996 Schoots et al., 1997
	KCNJ4	Kir2.3	22q13.1	Heart, brain, skeletal muscle	Blocker: Ba^{2+}		Makhina et al., 1994 Perier et al., 1994 Budarf et al., 1995
	KCNJ5	Kir3.4	11q24	Heart, pancreas	Blocker: Ba^{2+}, Cs^{+}		Tucker et al., 1995
	KCNJ6	Kir3.2	21q22.1-q22.2	Cerebellum, pancreatic islet	Blocker: Ba^{2+}, Cs^{+}	Mouse *weaver* Missense mutation (1 variant)	Lesage et al., 1994 Sakura et al., 1995 Patil et al., 1995 Tsaur et al., 1995
	KCNJ8	Kir6.1/ uK_{ATP}-1	12p11.23	Various	Blocker: Ba^{2+}, Cs^{+}		Inagaki et al., 1995b Inagaki et al., 1995c Erginel-Unaltuna et al., 1998
	KCNJ9	Kir3.3	1q21-23	Brain	Blocker: Ba^{2+}, Cs^{+}		Lesage et al., 1994 Kobayashi et al., 1995 Vaughn et al., 2000
	KCNJ10	Kir4.1	1q	Glia	Blocker: Ba^{2+}, Cs^{+}		Takumi et al., 1995 Tada et al., 1997
	KCNJ11	Kir6.2 (subunit of K_{ATP} channel)	11p15.1	Various		Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) a. Nonsense mutation (1 variant) b. Missense mutation (1 variant)	Inagaki et al., 1995a Thomas et al., 1995a Thomas et al., 1996a Nestorowicz et al., 1997
	KCNJ12	Kir2.2	17p11.2-p11.1	Atrium, ventricle	Blocker: Ba^{2+}, Cs^{+}		Wible et al., 1995 Hugnot et al., 1997 Namba et al., 1997
	KCNJ13	Kir7.1	2q37	GI, kidney, cerebellum, hippocampus, thyroid	Blocker: Ba^{2+}, Cs^{+}		Derst et al., 1998 Krapivinsky et al., 1998 Partiseti et al., 1998 Nakamura et al., 1999

TABLE 1
Continued

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms	References
	KCNJ14	Kir2.4	19q13	Brain, retina	Blocker: Ba^{2+}, Cs^+		Topert et al., 1998 Hughes et al., 2000 Topert et al., 2000
	KCNJ15	Kir4.2 Kir5.1	21q22.2	Kidney, lung, brain Brain, periphery			Gosset et al., 1997 Bond et al., 1994 Suzuki et al., 1999
Sulfonylurea receptor	*SUR1*	Sulfonylurea receptor 1 (subunit of K_{ATP} channel)	11p15.1	Pancreas, neurons, skeletal muscle	a. Blocker: glyburide, tolbutamide, glipizide, ciclazindol b. Opener: diazoxide	PHHI a. Missense mutations (11 variants) b. Deletion (4 variants)	Aguilar-Bryan et al., 1995 Thomas et al., 1995b Thomas et al., 1995a Nestorowicz et al., 1996 Thomas et al., 1996b Glaser et al., 1999 Dunne et al., 1997 Verkarre et al., 1998 Otonkoski et al., 1999 Tanizawa et al., 2000
	SUR2	Sulfonylurea receptor 2 (2A, 2B) (subunit of K_{ATP} channel)	12p12.1	2A: Heart, skeletal muscle 2B: Brain, liver, skeletal and smooth muscle	a. Blocker: glyburide, ciclazindole b. Opener: P1075, pinacidil, cromakalim		Chutkow et al., 1996 Inagaki et al., 1996 Isomoto et al., 1996
Large conductance Ca^{2+}-activated	*KCNMA1*	*Slo* (BK_{Ca} channel α-subunit)	10q23.1	Brain, smooth muscle, cochlea, pancreatic islets	a. Blocker: IBTX, CTX, TEA b. Opener: NS1619, NS8, BMS-204352, dehydrosoyasaponin I		Dworetzky et al., 1994 Pallanck and Ganetzky, 1994 Tseng-Crank et al., 1994 McCobb et al., 1995 Ferrer et al., 1996 Vogalis et al., 1996 Bowles et al., 2000
	KCNMB1	BK_{Ca} β1-subunit	5q34	Smooth and skeletal muscle			Knaus et al., 1994 Tseng-Crank et al., 1996 Jiang et al., 1999
	KCNMB2	BK_{Ca} β2-subunit		Kidney, heart, uterus, small intestine			Wallner et al., 1999a Uebele et al., 2000
	KCNMB3	BK_{Ca} β3-subunit	3q26.3-q27	Testis			Riazi et al., 1999 Behrens et al., 2000 Brenner et al., 2000 Uebele et al., 2000
	KCNMB4	BK_{Ca} β4-subunit	12q14.1-q15	Brain, heart, kidney, lung			Behrens et al., 2000 Brenner et al., 2000 Meera et al., 2000 Weiger et al., 2000
Small conductance Ca^{2+}-activated	*KCNN1*	SK1	19p13.1	Brain, heart	a. Blocker: tubocurarine, dequalinium, UCL 1848		Kohler et al., 1996 Litt et al., 1999 Shah and Haylett, 2000
	KCNN2	SK2		Brain, adrenal gland, Jurkat T cells	a. Blocker: apamin, ScTX, d-tubocurarine, 4-AP b. Opener: chlorzoxazone, zoxazolamine, 1-EBIO		Kohler et al., 1996 Jager et al., 2000 Syme et al., 2000
	KCNN3	SK3	1q21.3	Brain, heart, liver	Blocker: apamin		Kohler et al., 1996 Chandy et al., 1998 Wittekindt et al., 1998 Antonarakis et al., 1999 Austin et al., 1999

TABLE 1
Continued

Type	Gene	Nomenclature	Chromosome	Tissue Expression	Modulators	Disorder/Mechanisms	References
Intermediate conductance Ca^{2+}-activated	*KCNN4*	IKCa1	19q13.2	T lymphocytes, colon, smooth muscles, prostate, red blood cells, neurons, placenta, thymus	a. Blocker: CTX, clotrimazole, nitrendipine, miconazole, cetiedil, econazole, TRAM-34 b. Opener: 1-EBIO, chlorzoxazone, zoxazolamine		Ishii et al., 1997 Joiner et al., 1997 Logsdon et al., 1997 Ghanshani et al., 1998 Jensen et al., 1998 Neylon et al., 1999 Wulff et al., 2000
Two-pore K^+ channel	*KCNK1*	TWIK1	1q42-q43	Brain, kidney, heart	Blocker: Ba^{2+}, quinidine, quinine		Lesage et al., 1996a Lesage et al., 1996b Orias et al., 1997b
	KCNK2	TREK	1q41	Brain, lung	Potentiated by arachidonic acid, riluzole, chloroform, lysophosphatidylcholine, diethyl ether, halothane, isoflurane		Fink et al., 1996 Lesage and Lazdunski, 1998 Patel et al., 1999 Meadows et al., 2000 Duprat et al., 2000 Maingret et al., 2000
	KCNK3	TASK	2p23	Heart, brain, pancreas, placenta	a. Sensitive to external pH b. Activated by halothane, isoflurane		Duprat et al., 1997 Lesage and Lazdunski, 1998 Manjunath et al., 1999 Patel et al., 1999
	KCNK5	TASK2	6p21	Kidney	a. Sensitive to external pH b. Blocker: quinine, quinidine		Reyes et al., 1998
	KCNK6	TWIK2, TOSS	19q13.1	Eyes, lung, stomach, embryo	Sensitive to internal pH		Chavez et al., 1999 Gray et al., 1999 Pountney et al., 1999 Salinas et al., 1999
	KCNK7		11q13				Salinas et al., 1999
		TRAAK	11q13	Brain, spinal cord, retina	a. Blocker: gadolinium b. Opener: unsaturated fatty acid (arachidonic acid), riluzole, lysophosphatidylcholine c. Stretch-activated		Duprat et al., 2000 Lesage et al., 2000 Maingret et al., 2000
		CTBAK-1		Heart, brain, kidney	Blocker: Ba^{2+}		Kim et al., 1998

4-AP, 4-aminopyridine; AgTX2, angiotoxin 2; CTX, charybdotoxin; α-DTX, α-dendrotoxin; HgTX1, hongotoxin 1; IbTX, iberiotoxin; KTX, kaliotoxin; MgTX, margatoxin; NxTX, noxiustoxin; PaTX, phrixotoxin; ScTX, scyllatoxin.

is required to cause conformational changes leading to channel opening, which allows permeant ions to flow. The movement of this voltage sensor sensing changes in membrane potential has been monitored electrically as the gating current (Armstrong and Bezanilla, 1974). Mutational analysis and gating current measurements have suggested that the transmembrane S4 segment represents the major component of the voltage sensor (Papazian et al., 1991; Perozo et al., 1994). The S4 segment that contains positively charged residues (lysine or arginine) at approximately every third position resulting in a regularly spaced array of 5 to 7 positive charges is conserved within the voltage-gated K^+ channel family. The rearrangement of S4 in response to membrane depolarization has also been confirmed by the means of fluorescence techniques (Mannuzzu et al., 1996; Cha and Bezanilla, 1997). While the S4 segment comprises the major part of the voltage sensor required for the K^+ channel activation, the electrostatic interaction of negative charges in S2 and S3 with the S4 segment also contributes to the gating mechanism (Papazian et al., 1995; Seoh et al., 1996). The nature of the gate that ultimately controls access of permeant ions to the pore is not conclusively established. Studies involving mutational analysis, gating current measurements, and the substituted cysteine accessibility method point to several residues in the S5 and S6 segments that might form the activation gate regulating access of ions to the pore (Liu et al., 1997; Shieh et al., 1997; Kanevsky and Aldrich, 1999).

c. Inactivation. Many voltage-dependent K^+ channels activate and inactivate rapidly when membrane potential becomes more positive. Inactivation is a nonconducting state during maintained depolarization. Three types of inactivation, i.e., N-, P-, and C-type, have been characterized and associated with distinct molecular domains of the channel. For example, the N-terminal residues (amino acids 6–46) of the *Shaker* K^+ channel involved in N-type inactivation moves into the internal vestibule (in S4-S5 linker) to occlude the pore when the channel opens (Hoshi et al., 1990; Isacoff et al., 1991). After removal of this N terminus region, inactivation can be restored in the mutant K^+ channel by the corresponding synthetic peptide (Zagotta et al., 1990). In contrast to the fast process of N-type inactivation, the C- and P-type inactivation involves a slower rearrangement of outer mouth and specific residues in the pore, respectively (Hoshi et al., 1991; De Biasi et al., 1993; Yellen et al., 1994; Liu et al., 1996).

d. Subunit Interaction and Assembly Domains. As noted previously, K^+ channels contain four α-subunits, which surround a water-filled, K^+-selective pore (Fig. 2). Among diverse voltage-gated K^+ channels, only closely related subfamilies of α-subunits are capable of coassembling to form heteromultimers. For example, in the Kv1 subfamily, a highly conserved cytoplasmic sequence immediately preceding the first transmembrane segment (amino acid residues 83 to 196) was identified as important to subfamily-specific channel assembly (Li et al., 1992). In *Shaker* channels, a conserved region (T1, or tetramerization domain 1) in the first transmembrane segment is involved in formation of tetramers (Shen et al., 1993). However, in more distantly related voltage-gated K^+ channels, *ether-a-go-go* (EAG), hERG, and KCNQ1 K^+ channel subfamilies, channel assembly primarily involves C-terminal domains (Ludwig et al., 1997; Kupershmidt et al., 1998). As discussed in the following sections, patients with Jervell and Lange-Nielsen long-QT (LQT) syndrome are characterized by the absence of KCNQ1 heteromultimers caused by mutations in the C terminus that impair subunit assembly (Schmitt et al., 2000).

2. Two Transmembrane One-Pore Channels. The inward rectifier K^+ channels (Kirs) belong to a distant superfamily of channels with four subunits each containing a two-transmembrane segment (M1 and M2) and a pore loop in between (Ho et al., 1993; Kubo et al., 1993). These channels conduct K^+ currents more in the inward direction than outward, and they are important in setting the resting membrane potential. This inward rectification is attributed to gating mechanisms by internal Mg^{2+} and polyamines (spermine, spermidine, etc.) that occlude access of K^+ to the internal vestibule of a conducting pore (Matsuda, 1991; Ficker et al., 1994; Lu and MacKinnon, 1994; Wible et al., 1994). Like the voltage-gated K^+ channels, these channels are organized as tetramers (Yang et al., 1995), although a more complex octameric arrangement has been described, as in the case of the ATP-sensitive K^+ channels involving four inward rectifiers contributing to ion conducting pore and four peripheral sulfonylurea receptors as regulatory subunits (Clement et al., 1997; Inagaki et al., 1997; Shyng and Nichols, 1997).

3. Four Transmembrane Two-Pore Channels. The more recently discovered tandem-pore domain family are weak inward rectifiers with four putative transmembrane domains and two pore domains (Ketchum et al., 1995; Lesage et al., 1996a). They represent perhaps the most abundant class of K^+ channels (at least in *C. elegans*), with >50 distinct members (Wang et al., 1999). The G(Y/F)G residues of K^+-selective motif is preserved in the first pore loop of the two-pore K^+ channel, but it is replaced by GFG or GLG in the second pore loop. Although all the two-pore channels have a conserved core region between transmembrane segments M1 and M4, the amino- and carboxyl-terminal domains are quite diverse. With two-pore domain subunits, two such subunits would presumably form a channel to retain the tetrameric arrangement.

B. Auxiliary Subunits

Auxiliary subunits that associate with many of the pore-forming subunits have also been described (reviewed in Isom et al., 1994). For example, the Kv1 channels associate

with cytoplasmic β-subunits to alter channel kinetics (reviewed in Xu and Li, 1998). More recently, chaperone proteins, such as KChAP, regulating the function and expression of some of the Kv channels, such as Kv2.1, Kv1.3, and Kv4.3, have been reported (Kuryshev et al., 2000b). Certain other Kv channels, such as Kv5, Kv6, Kv8, and Kv9, do not form functional channels themselves but associate with Kv2.1 channels to alter the biophysical properties (Salinas et al., 1997; Kramer et al., 1998). Other examples include distinct β-subunits that associate with the calcium-activated K^+ channels (Tseng-Crank et al., 1996; Wallner et al., 1999a; Behrens et al., 2000; Brenner et al., 2000; Meera et al., 2000), sulfonylurea receptors for the inward rectifiers Kir6.1 or Kir6.2 (Aguilar-Bryan et al., 1995; Inagaki et al., 1995a), and minK and minK-related peptides (MiRPs) for the cardiac delayed rectifier channels (Barhanin et al., 1996; Sanguinetti et al., 1996; Abbott et al., 1999). These subunits play roles as diverse as modulation of gating properties such as inactivation, cell surface expression, and/or trafficking of the ion channel complex, to serving as binding sites for both endogenous and exogenous ligands. Given the diversity of K^+ channel subunits and the potential to vary the constituents to form diverse α-α or α-β heteromeric channel complexes to alter expression, cellular targeting, and biophysical and pharmacological properties in native cell types, understanding the precise composition of channel complexes in vivo remains a challenge.

C. Crystal Structure of K^+ Channels

Initial studies of the structure and function of K^+ channels by a combination of mutagenesis and biophysical approaches have revealed domains that are responsible for K^+ selectivity, gating, channel assembly, subunit interaction, and drug binding sites. However, the three dimensional structural implications remained largely speculative. Recent discovery of the crystal structure of the KCsA channel established a blueprint of K^+ channel structure with 3.2 Å resolution (Doyle et al., 1998). The KCsA channel is encoded by a bacterial gene cloned from *S. lividans* on the basis of sequence homology to K^+-selective motif GYG in the P-loop (Schrempf et al., 1995). The KCsA channel contains only two transmembrane domains with an intervening pore loop, although at the amino acid sequence level, this channel is more similar to the voltage-gated K^+ channels. Functionally, it lacks any hint of voltage gating because of the lack of S4 region. X-ray analysis revealed that four identical subunits form a tetramer creating an inverted cone, cradling the selectivity filter of the pore in its outer end. The overall length of the conducting pore is 45 Å, and its diameter is variable along its distance. The internal vestibule of the pore begins as a tunnel of 18 Å in length that widens into a cavity (~10 Å across) near the middle of the membrane, with the narrow selectivity filter only 12 Å long. The remainder of the pore is wider and lined with hydrophobic amino acids. The selectivity filter is lined by the carbonyl oxygen atoms of the GYG signature sequence, which is held open by structural constraints to coordinate K^+ ions (~3 Å) but not smaller Na^+ ions because the diameter is too wide to substitute for the hydration energy of the Na^+ ions (Doyle et al., 1998). The crystal structure of KCsA channel provides the first three-dimensional structure of the conduction pore that fits consistently with current understanding of the core functionality of K^+ channels. However, structural information of the remaining transmembrane segments (S1-S4), particularly the voltage sensor and the gate coupling to channel opening and closing, remains to be elucidated. Nevertheless, the understanding of structural information can be applied to design selective compounds targeting K^+ channels. For example, a structure-based design strategy allowed several charybdotoxin analogs to be prepared with about 20-fold higher affinity to block Ca^{2+}-activated K^+ channels versus voltage-gated Kv1.3 channels (Rauer et al., 2000). It is to be anticipated that a detailed understanding of the structural aspects would revolutionize and refine approaches targeting K^+ channels for therapeutic purposes.

II. Pathophysiologic Regulation of K^+ Channels: Genetically Linked Diseases

Advances in genetic linkage analysis during the past decade have greatly facilitated the identification of many disease-producing loci. Both positional cloning and candidate gene approaches have been used. Using positional cloning techniques, it has become possible to identify the location of genetic locus responsible for a given hereditary syndrome without prior knowledge of the biochemical or physiological abnormalities underlying the disease. Alternatively, following identification of genes encoding proteins that may be logically altered in a particular disease, the candidate gene approach may be used to examine genetic linkage to the hereditary disease of interest and screened for mutations.

As K^+ channels play fundamental roles in the regulation of membrane excitability, it is to be expected that both genetic and acquired diseases involving altered functioning of neurons, smooth muscle, and cardiac cells could arise subsequent to abnormalities in K^+ channel proteins. Genetically linked diseases of the cardiac, neuronal, renal, and metabolic systems involving members of voltage-gated K^+ channels, inward rectifiers, and channel-associated proteins are discussed in the following sections (Table 1).

A. Cardiac Diseases

K^+ channels are critical to cardiac excitability because they play a fundamental role in repolarization of the action potential. Unlike the action potentials of nerves that last only a few milliseconds, the action potentials of ventricular myocytes can last several hundred milliseconds. This prolonged depolarization phase is essential

for normal excitation-contraction coupling process and renders the myocytes relatively refractory to premature excitation. Various classes of K^+ channels with different time and voltage dependencies and pharmacological properties function in concert to regulate the heart rate by setting the resting membrane potential, amplitude, and duration of action potential and its refractoriness (Barry and Nerbonne, 1996; Roden and Kupershmidt, 1999; Snyders, 1999). The Kir2.1 current sets the resting membrane potential and contributes to the terminal phase of repolarization. The transient outward K^+ current (Kv4.3 or Kv1.4), which is Ca^{2+}-independent and expressed in a species- and cell type-specific fashion, is important for the early phase of repolarization. The long ventricular action potentials that result from the slow onset of repolarization are controlled mainly by two types of delayed rectifier K^+ currents, i.e., IKs (derived from KCNQ1/minK) and IKr (derived from hERG/MiRP1). Both genetic linkage analysis and the candidate gene approach revealed that mutations in these delayed rectifier K^+ channel subunits form the molecular basis of LQT syndromes (Curran et al., 1995; Sanguinetti et al., 1995; Schott et al., 1995; Wang et al., 1996; Neyroud et al., 1997; Splawski et al., 1997b; Abbott et al., 1999).

The LQT syndromes are inherited genetic disorders characterized by prolonged or delayed ventricular repolarization, manifested on the electrocardiogram (ECG) as a prolongation of the QT interval. Table 2 lists K^+ and other ion channel genes involved in various forms of inherited LQT syndromes, LQT1 through LQT6. The inherited LQT causes syncopal attacks and high risk of sudden death as result of torsade de pointes polymorphic ventricular tachycardia, typically triggered by adrenergic arousal (Ackerman and Clapham, 1997; Sanguinetti and Spector, 1997; Vincent et al., 1999). Based on genetic origins, two allelic diseases are recognized: 1) the Romano-Ward syndrome inherited as a dominant trait and 2) the autosomal recessive Jervell and Lange-Nielsen syndrome. In the case of the latter, the patient suffers from a severe congenital bilateral deafness in addition to the cardiac disorder (Vincent et al., 1999). Note that in addition to genetically linked LQT syndromes, many drugs are also known to cause QT prolongation leading to torsade de pointes (see *Section III.*).

TABLE 2
K^+ channel genes involved in long-QT syndromes

Type	Gene	Current/Channel Type
LQT1	KCNQ1 (KvLQT1)	Component of slowly inactivating delayed rectifier IKs
LQT2	hERG	Delayed rectifier IKr rapidly inactivating cardiac Na^+ channel
LQT3	SCN5A	
LQT4	Chromosome 4q25-27	Subunit involved in regulation of cardiac repolarization?
LQT5	KCNE1 (MinK)	Component of IKs
LQT6	MiRP1	Component of IKr

1. Long-QT1 and Long-QT5 Syndromes: KCNQ1 (KvLQT1) and minK. KvLQT1, encoded by the KCNQ1 gene, in association with the minK subunit, a short peptide of 130 residues, constitutes the IKs responsible for phase 3 repolarization in the heart (Barhanin et al., 1996; Sanguinetti et al., 1996b). Several mutations in the *KCNQ1* gene, including missense mutations, intragenic deletion, and insertions, are involved in chromosome 11-linked LQT1 syndrome, the most common form of inherited LQT in families with Jervell and Lange-Nielsen and Romano-Ward syndromes (Russell et al., 1996; Wang et al., 1996; Donger et al., 1997; Tanaka et al., 1997; van den Berg et al., 1997; Saarinen et al., 1998; Li et al., 1998; Neyroud et al., 1999;). Functional analysis of mutant channels in COS cells cotransfected with the minK subunit revealed that these mutations either alter gating properties or fail to produce functional homomeric channels and reduced K^+ current when coexpressed with the wild-type subunit (Chouabe et al., 1997; Shalaby et al., 1997; Wollnik et al., 1997; Franqueza et al., 1999).

Two separate mutations (D76N and S74L) in the minK subunit were identified in patients phenotypically characterized with LQT5 syndrome by single strand conformation polymorphism analyses (Splawski et al., 1997b; Duggal et al., 1998). Again, functionally, these mutations yield diminished IKs current when coinjected with *KCNQ1* either by suppressing channel function in a dominant-negative fashion, increasing rate of channel deactivation, or by shifting the voltage dependence of channel activation in a positive direction. It is likely that the mutations in *KCNQ1* associated with LQT1 will decrease the availability of IKs by altering gating properties or by a dominant-negative loss of channel function leading to a prolonged ventricular repolarization. Accordingly, activators that restore the function of IKs may prove useful in the treatment of LQT1 and LQT5 syndromes. Recently, Abitbol et al. (1999) have shown that stilbenes and fenamates, by binding the extracellular domain flanking the minK transmembrane segment, restored inactive IKs mutant channels, including the naturally occurring LQT5 mutant, D76N.

Neyroud et al. (1997) have also identified a homozygous deletion-insertion event in the C-terminal domain of *KCNQ1* in three affected children from two families with congenital bilateral deafness associated with QT prolongation. By in situ hybridization studies in mice, it was shown that the *KCNQ1* gene was expressed by the marginal cells of the stria vascularis. It has been suggested that, in conjunction with the minK subunit, KCNQ1 forms a functional channel in marginal cells that is responsible for secretion of endolymph, in the inner ear, which bathes the stereocilia of sensory hair cells. Thus, KCNQ1 plays a key role not only in the ventricular repolarization but also in normal hearing, probably via control of endolymph homeostasis (see *Section II.C.*).

2. Long-QT2 Syndrome and Human ether-a-go-go-Related K^+ Channel. The hERG gene encoding a rapidly activating IKr is a major subunit responsible for repolarization during cardiac action potential (Sanguinetti et al., 1995). Interaction with hERG channels has been shown to be a primary mechanism involved in the therapeutic actions of the class III antiarrhythmic agents and the potential cardiotoxicity of second generation H_1 receptor antagonists, such as terfenadine and astemizole, as well as certain antidepressants and neuroleptics (Vincent et al., 1999).

By linkage analysis and single strand conformation polymorphism, Curran et al. (1995) first demonstrated that missense mutations, intragenic deletions, and splice donor mutations in the hERG gene resulted in chromosome 7-linked LQT2 syndrome. This finding was further confirmed by studying several mutations in different regions of the hERG subunit in families associated with LQT syndromes (Benson et al., 1996; Dausse et al., 1996; Satler et al., 1996, 1998; Tanaka et al., 1997). Similar to KCNQ1, mutations of hERG decrease repolarizing current and thus lengthen the duration of cardiac action potential. The mutant hERG cRNA, when expressed alone or in combination with wild-type channel, yields nonfunctional channels or evokes dominant negative suppression of hERG function (Sanguinetti et al., 1996a; Li et al., 1997; Babij et al., 1998; Nakajima et al., 1998). By green fluorescent protein tagging and Western blot analyses, it was found that the hERG-G601S mutant was deficient in the trafficking of functional protein to the plasma membrane (Furutani et al., 1999), which could explain the reduction in functional channels available for repolarization of the cardiac action potential. Other LQT-associated mutations identified in the amino-terminal region of hERG form functional channels, but with altered gating properties such as accelerated channel deactivation, and positively shifted voltage dependence of channel open probability. Collectively, these alterations lead to reduced outward current during the repolarization phase of the cardiac action potential and prolonged QT interval (Chen et al., 1999a). The diversity of mutations in the hERG gene impairing channel function in varying proportions likely contributes to variable degrees of clinical severity in LQT2 patients.

Although channels formed of hERG subunits appear similar to IKr, and although mutations in hERG gene are associated with LQT2 syndrome, the recombinant channels differ in gating, single channel conductance, and sensitivity to antiarrhythmic drugs compared with native currents. Another small membrane subunit, MiRP1, cloned by searching the expressed sequence tag (EST) database, was found to assemble with hERG to alter its function (Abbott et al., 1999). Injection of MiRP1 cRNA alone into oocytes revealed no currents by itself, whereas MiRP1 had significant effects on the properties of channels formed with hERG subunits but not with other K^+ channels, including KCNQ1, *Shaker*, and Kv members. Coexpression of MiRP1 with hERG revealed functional current with gating and sensitivity to E-4031 similar to native cardiac IKr. Three missense mutations associated with the LQT6 syndrome and ventricular fibrillation have been identified in the MiRP1 gene. The mutant channels open slowly and close rapidly, thereby evoking diminished K^+ currents. One variant, associated with clarithromycin-induced arrhythmia, increases sensitivity to channel blockade by the antibiotic. The latter finding reveals an important mechanism for acquired arrhythmia wherein a genetically based reduction in K^+ currents remains silent until combined with additional factors.

β-Adrenoceptor antagonists have been used in the treatment of LQT1 and LQT2 syndromes since episodes of syncope and sudden death occur more frequently with exercise and at times of adrenergic surges (Vincent et al., 1999). The mechanism of dysfunction of hERG and MiRP1 associated with LQT suggests that activators for these channels may be therapeutically useful. Expression of hERG alone reveals little outward K^+ current upon depolarization, whereas large inward K^+ currents are seen when the membrane voltage is hyperpolarized due to removal of C-type inactivation (Smith et al., 1996; Spector et al., 1996). Elevation of external K^+ levels reduces this C-type inactivation, thereby increasing outward K^+ currents and reducing the prolongation of cardiac action potential with LQT2. Indeed, Compton et al. (1996) have shown that elevation of serum $[K^+]$ using K^+ supplements and spironolactone in patients with LQT2 demonstrated a significant reduction of the QT interval. Although it is difficult to maintain an elevated level of serum K^+, these findings suggest that the patients could avoid administration of drugs that cause hypokalemia.

B. Neuronal Diseases

K^+ channels are critical to neurotransmission in the nervous system. Alterations in the function of these channels lead to remarkable perturbations in membrane excitability and neuronal function. Significant progress has been made in linking many neuronal disorders, including episodic ataxia and benign familial neonatal convulsions, to K^+ channel mutations.

1. Episodic Ataxia/Myokymia and Kv1.1. Episodic ataxia (EA) is an autosomal dominant disorder in which the affected individuals have brief episodes of ataxia triggered by physical or emotional stress. On the basis of the duration and severity of the attacks, two types of episodic ataxia are recognized. In EA type 1 with onset in early childhood, the ataxia occurs several times during the day, lasts for seconds to minutes, and is associated with dysarthria and motor neuron activity, which causes muscle rippling (myokymia) between and during attacks. In contrast, in EA type 2, the attacks last for hours to several days and are precipitated by emotional

stress and exercise, but they do not startle. This type of ataxia is associated with nystagmus and cerebellar atrophy, unlike the EA-1 type in which the affected children do not develop persistent ataxia or cerebellar atrophy.

Linkage analysis has mapped episodic ataxia to two different ion channel genes. EA-2 is associated with missense mutations in *CACNA1A*, encoding a brain-specific P/Q-type Ca^{2+} channel located on chromosome 19p13, the same region associated with familial hemiplegic migraine, suggesting the possibility that both EA-2 and familial hemiplegic migraine are allelic disorders (Ophoff et al., 1996; Jen et al., 1999). By linkage studies, Litt et al. (1994) localized the EA-1 gene to chromosome 12p, where the *KCNA1* gene encoding the voltage-gated K^+ channel in brain and peripheral nervous systems has been mapped. Mutational analysis of *KCNA1* in several families with EA-1 has identified at least ten missense mutations (Browne et al., 1994; Scheffer et al., 1998). These mutations alter Kv1.1 function by reducing channel expression (dominant-negative effect), altering gating properties by shifting the midpoint of current activation some 10 to 40 mV in the depolarization direction, or enhancing deactivation or C-type inactivation rates (Adelman et al., 1995; Zerr et al., 1998; Boland et al., 1999; Bretschneider et al., 1999). Accordingly, it could be inferred that altered Kv1.1 function could impair the capacity of the affected neurons to repolarize effectively following an action potential. Further support for the notion that the diminished function of *KCNA1* leads to ataxia is obtained from gene knockout studies in which the homozygous mutant mice exhibit attacks of tremors and marked ataxia after cold-temperature stress (Smart et al., 1998).

Acetazolamide, a carbonic anhydrase inhibitor, has been effective in reducing attack episodes in some patients suffering from EA-1. However, this compound did not affect Kv1.1 wild-type or mutant channels (Bretschneider et al., 1999). Pharmacological agents that either shift the voltage dependence of Kv1.1 channel activation to more negative potentials or enhance the magnitude of current could, in principle, prevent both ataxia and myokymia (Sanguinetti and Spector, 1997).

2. Benign Familial Neonatal Convulsions and KCNQ2/KCNQ3. Recent application of genetic analysis to hereditary epilepsy has provided the impetus for the identification of mutations in genes encoding various ion channels, including K^+ channels (Biervert et al., 1998; Charlier et al., 1998; Singh et al., 1998). BFNC is an idiopathic form of epilepsy beginning within the first six months after birth. Seizures are generalized and mixed, starting with tonic posture, ocular symptoms, and apnea, and often progress to clonic movements and motor automatisms. Seizures last 1 to 2 s and occur three to six times per day. Two forms of benign familial neonatal convulsions, BFNC1 and BFNC2, are typically observed in families as an autosomal dominant inheritance and have been previously mapped into chromosomes 20q and 8q, respectively (Leppert and Singh, 1999). By positional cloning techniques, the voltage-gated K^+ channel *KCNQ2*, spanning the deletion region of chromosome 20q13.3 that cosegregates with seizures in a BFNC family, was identified (Biervert et al., 1998; Singh et al., 1998). Missense mutation, frameshifts, and splice-site mutations in *KCNQ2* were also found in other BFNC families. By a homology search of expressed sequence tag database and genotyping approaches, a missense mutation in the pore region of another voltage-gated K^+ channel, *KCNQ3*, was also identified from families with BFNC2 previously linked to chromosome 8q24 (Biervert et al., 1998; Charlier et al., 1998; Schroeder et al., 1998).

It is now understood that both *KCNQ2* and *KNCQ3* subunits coassemble to constitute properties of the M-channel (M for *muscarine*) described in neurons (Brown and Adams, 1980). First described in the peripheral sympathetic neurons and subsequently in the CNS, this channel is one of the most important regulators of neuronal excitability because it plays a critical role in determining the excitability threshold, firing properties, and responsiveness of neurons to synaptic inputs. In the absence of acetylcholine, the M-channel activity hyperpolarizes the cell membrane potential, leading to a dampening of neuronal responsiveness to synaptic inputs. However, in the presence of released acetylcholine, the M-channels are inhibited. This change in M-channel activity provides a mechanism for neurons to respond to synaptic input and to favor firing a burst of spikes, rather than a single spike, upon excitation (Hille, 1992). By voltage-clamp recording of neurons from rat superior cervical ganglion, Marrion et al. (1989) determined that ACh-evoked suppression of the M-channel is mediated by the activation of muscarinic M1 receptors. Seizures in mice induced by a muscarinic agonist, pilocarpine, were sensitive to inhibition by a muscarinic M1 antagonist, pirezepine (Maslanski et al., 1994). Furthermore, in transgenic mice lacking muscarinic M1 receptors, the robust suppression of the M-current activity evoked by muscarinic agonists in sympathetic ganglion neurons was eliminated. Both homozygous and heterozygous mutant mice were also resistant to seizures evoked by systemic administration of pilocarpine (Hamilton et al., 1997). Taken together, these studies suggest that M-channels play a key role in controlling seizure activity.

Both *KCNQ2* and *KCNQ3* belong to the *KCNQ* family of K^+ channels that includes *KCNQ1* (KvLQT1), whose aberrant function leads to the congenital bilateral deafness associated with QT prolongation. The KCNQ2 protein exhibits 62% identity with KCNQ3 within the coding region and is also highly conserved with KCNQ1 in transmembrane S1-S6 region with 60% identity and 70% similarity (Biervert et al., 1998; Charlier et al., 1998; Tinel et al., 1998). Unlike *KCNQ1*, which is expressed strongly in human heart and pancreas, *KCNQ2*

and *KCNQ3* transcripts are detectable only in brain (Biervert et al., 1998, Wang et al., 1998; Yang et al., 1998) and in rat sympathetic ganglia (Wang et al., 1998). Expression of human *KCNQ2* was found to be high in the hippocampus, caudate nucleus, and amygdala, moderate in the thalamus, and weak in the subthalamic nucleus, substantia nigra, and corpus callosum. A similar expression pattern for *KCNQ3* was found in the human brain (Biervert et al., 1998; Tinel et al., 1998; Yang et al., 1998).

In human brain, four splice variants of KCNQ2 were identified, among which only two forms generated K^+-selective currents when heterologously expressed in oocytes or COS cells (Tinel et al., 1998). These currents resemble those of KCNQ1 in their permeability sequence of cations, voltage dependence, and kinetics (Biervert et al., 1998; Tinel et al., 1998). When expressed in *Xenopus* oocytes, KCNQ3 elicited currents that were only slightly above background but resembled the larger depolarization-activated K^+ currents observed with KCNQ2 (Schroeder et al., 1998; Wang et al., 1998). Unlike *KCNQ1* (KvLQT1), where coinjection with minK (*KCNE1*) dramatically alters the amplitude and gating kinetics of the KCNQ1 channel and produces current resembling cardiac IKs, neither KCNQ2 or KCNQ3 currents were altered when coinjected with the minK subunit (Yang et al., 1998). However, when *KCNQ2* and *KCNQ3* mRNAs were coinjected in the *Xenopus* oocytes, the resultant current was more than 10-fold larger than that observed in cells injected with either *KCNQ2* or KCNQ3 alone (Schroeder et al., 1998; Wang et al., 1998; Yang et al., 1998). The expressed K^+ current by coinjection with *KCNQ2* and *KCNQ3* has gating kinetics and sensitivities to blockade by classical M-channel inhibitors such as linopirdine and XE991, indicating that the M-channel is a heteromultimer composed of KCNQ2 and KCNQ3 subunits (Wang et al., 1998).

No detectable currents were expressed when cRNA of the truncated *KCNQ2* identified from families with BFNC1 alone were injected. When mutant and wild-type cRNA were coinjected at a 1:1 ratio to mimic the situation in a heterozygous patient, the currents were reduced, compared with those recorded from oocytes injected with similar amounts of wild-type cRNA. Thus, although there was no obvious dominant negative effect, haploinsufficiency may be enough to explain the dominant mode of inheritance of this disorder, which generally occurs transiently during infancy (Biervert et al., 1998). Two single mutations in *KCNQ2* (Y284C and A306T), as well as insertion mutant associated with BFNC1, were analyzed for current amplitude when coexpressed with *KCNQ3*. The function of these mutant heteromeric channels was significantly reduced, and no dominant negative effect was observed. Likewise, when the *KCNQ3* mutant G310V was coexpressed with wild-type *KCNQ2*, a loss function effect rather than a dominant-negative effect was seen (Schroeder et al., 1998). Together, Schroeder et al. (1998) suggested that a 25% loss of heteromeric KCNQ2/KCNQ3 function is sufficient to cause the hyperexcitability in BFNC. Recently, another missense mutation that replaced tryptophan with arginine (W309R) in the P-loop of KCNQ3 was also reported from patients with BFNC (Hirose et al., 2000).

The cytoplasmic N terminus of *KCNQ2* contains a consensus site for cAMP-dependent phosphorylation, and increases in intracellular cAMP concentration have been shown to enhance KCNQ2/KCNQ3 current by 50% (Schroeder et al., 1998). Compounds that open or enhance the activity of the M-currents, such as retigabine, or elevate associated intracellular cAMP levels may serve as useful antiepileptic agents. It should be pointed out that in addition to M-channel mutations linking to BFNC disorders, mutations in other ion channels have been associated with varying forms of epilepsy (Steinlein, 1999). These include mutations of the neuronal nicotinic acetylcholine receptor α4-subunit (*CHRNA4*), identified to be responsible for the autosomal dominant nocturnal frontal lobe epilepsy (Steinlein et al., 1995, 1997), and those involving voltage-gated sodium channel α1-subunit (*SCN1B*) identified in families associated with generalized epilepsy with febrile seizures (Wallace et al., 1998). Collectively, the discoveries of these ion channels as epilepsy disease genes emphasize the potential roles of ion channels in epilepsy and suggest that compounds that directly or indirectly modulate these channels may prove helpful in suppressing seizures.

3. Neurodegeneration and Kir3.2. The progressive loss of dopaminergic neurons in the *weaver* mouse is similar to the pathological symptom of Parkinson's disease where cell death of dopaminergic neurons in the substantia nigra is observed, leading to striatal dopaminergic deficit and a clinical syndrome dominated by disorders of movement (Yamada et al., 1990; Gaspar et al., 1994). The *weaver* phenotype in mice is an autosomal recessive neurological and reproductive disorder characterized behaviorally by severe ataxia, hyperactivity, and tremors that are manifested within 2 weeks after birth. These behavioral changes are attributable to the degeneration of cerebellar granule cells and dopaminergic neurons in the substantia nigra (Rakic and Sidman, 1973a,b). In addition, *wv/wv* genotype causes death or impaired function of dopaminergic neurons in the substantia nigra, male infertility, and sporadic tonic-clonic seizures (Hess, 1996; Harrison and Roffler-Tarlov, 1998). While heterozygous mice are not ataxic, they have seizures and a significant reduction in the number of granule cells.

The *weaver* mutation was mapped to mouse chromosome 16 in a region of conserved linkage with human chromosome 21 (Reeves et al., 1989). By a combination of physical and transcript mapping of the homologous segment on human chromosome 21, Patil et al. (1995) identified two potential candidate genes in this region: 1) *mmb*, encoding a serine/threonine-specific protein

kinase, and 2) Kir3.2, encoding a G protein-gated inwardly rectifying K^+ channel. Sequence analysis yielded no mutations in *mmb*, whereas a single missense mutation replacing a glycine with serine at residue 156 (G156S) was observed in Kir3.2 associated with *weaver* mouse in a location within the pore-forming region, critical for ion selectivity and conserved within the K^+ channel family (MacKinnon, 1995). The mutation renders the channel nonselective, leading to conduction of Na^+ ions instead of the highly selective K^+ ions (Navarro et al., 1996; Slesinger et al., 1996). Ribonuclease protection and reverse transcriptase-polymerase chain reaction studies have shown that the overall expression pattern of Kir3.2 gene parallels the developmental loss of the cells in cerebellum, substantia nigra, and testes (Patil et al., 1995; Slesinger et al., 1996).

It has been shown that Kir3.2 coassembles with Kir3.1 to form the G protein-gated, K^+-selective inward rectifier channels in neurons (Duprat et al., 1995; Liao et al., 1996; Velimirovic et al., 1996). Immunohistochemical localization studies indicate that Kir3.2 and Kir3.1 proteins are expressed in the cerebellar neurons of mice at postnatal day 4, at a time when neurons normally undergo differentiation (Slesinger et al., 1996). Functional analysis of expression of *wv*Kir3.2 and Kir3.2 in *Xenopus* oocytes or Chinese hamster ovary cells revealed that the mutant channel showed reduced sensitivity to muscarinic M2 receptor activation, failed to respond to G_α subunit, and evoked diminished K^+ currents. Furthermore, the loss in selectivity for K^+ and increased basal current resulting from increased Na^+ permeability leads to alterations in membrane excitability, cell differentiation, and ultimately cell death (Kofuji et al., 1996; Navarro et al., 1996; Silverman et al., 1996; Slesinger et al., 1996; Rossi et al., 1998). Results from transgenic studies confirmed that the *weaver* phenotypes arise from a gain-of-function mutation of Kir3.2. Although the transgenic mice lacking Kir3.2 (−/−) are morphologically indistinguishable from the wild type, they have much reduced Kir3.1 expression in the brain, develop spontaneous seizures, and are more susceptible to pharmacologically induced seizures induced by pentylenetetrazol (Signorini et al., 1997).

The nonselective cation current in cells expressing *wv*Kir3.2 can be blocked by MK-801 and calcium channel blockers (Kofuji et al., 1996). These compounds have been shown to enhance cell viability and neurite outgrowth of cultured *weaver* granule cells, but not of wild-type granule cells. In addition, neurite outgrowth and migration of the *weaver* granule neurons has also been shown to be enhanced by Fab2 fragments of antibodies raised against a neurite outgrowth domain of the laminin B2 chain (Liesi and Wright, 1996).

As mentioned previously, the degeneration of noncalbindin-positive dopaminergic neurons in substantia nigra of *weaver* mice shares similarity to Parkinson's disease, in which the dopaminergic neurons that are progressively lost in the substantia nigra are also noncalbindin-positive. These observations suggest the possibility of a shared genetic defect in *weaver* mouse and Parkinson's disease (Yamada et al., 1990; Gaspar et al., 1994). However, Bandmann et al. (1996) did not detect mutations by sequencing analysis of the pore-forming region of Kir3.2 gene from patients with familial and sporadic cases of Parkinson's disease, suggesting a differing etiology of nigral cell loss in Parkinson's disease and *weaver* mice. Nevertheless, the finding that *weaver* phenotype results from a single amino acid mutation in Kir3.2 leading to alterations in membrane excitability provides a reasonable avenue for understanding the molecular nature of this neuronal disorder.

4. Schizophrenia and SK3 (hKCa3). Although initially differentiated on the basis of biophysical and differential toxin sensitivity, distinct genes are now known to encode various calcium-activated K^+ channels (Vergara et al., 1998; Castle, 1999; Wallner et al., 1999b). Abnormal function of calcium-activated K^+ channels has been noted in platelets of patients with Alzheimer's disease, although its relevance to the pathology is not clear (de Silva et al., 1998). The CAG triplet repeat in *KCNN3* gene encoding a small conductance calcium-activated K^+ channel, hKCa3, mapped to chromosome 1q21 has been reported to be associated with schizophrenia (Chandy et al., 1998), although subsequent investigations to confirm these findings have been met with mixed results (Austin et al., 1999; Dror et al., 1999).

C. Hearing and Vestibular Diseases: Nonsyndromic Dominant Deafness and KCNQ4

Much progress has been made in the area of identifying genes defective in hearing and balance disorders, with over 40 such genes described (Holt and Corey, 1999). One of the genes reported to be the locus for hereditary hearing impairment is another K^+ channel belonging to the *KCNQ* channel superfamily, i.e., *KCNQ4*. The *KCNQ4* gene, isolated from a human retina library using *KCNQ3* partial cDNA as a probe, exhibits 38, 44, and 37% identity to *KCNQ1*, *KCNQ2*, and *KCNQ3*, respectively (Kubisch et al., 1999). Reverse transcriptase-polymerase chain reaction analysis revealed high expression of *KCNQ4* in the vestibular system and brain. In cochlea sections from mice at postnatal day P12, sensory outer hair cells were strongly labeled with a *KCNQ4* antisense probe but not in the inner hair cells and stria vascularis where *KCNQ1* expression was detected. Expression of *KCNQ4* in *Xenopus* oocytes generated a voltage-dependent K^+ current, similar to *KCNQ1, KCNQ2*, and *KCNQ3*, except with slower activation. Unlike *KCNQ1, KCNQ4* did not interact with minK. However, coexpression of *KCNQ3* with *KCNQ4* yielded currents resembling an M-channel, but with only weak inhibition (75% inhibition at 200 μM) by linopirdine, unlike those observed with the *KCNQ2/KCNQ3* combination. The similarity of currents from

KCNQ3/KCNQ4 to M-channel indicated that *KCNQ3/KCNQ4* might potentially form another M-channel variant in the nervous system (Kubisch et al., 1999).

Using fluorescence in situ hybridization to human chromosomes, *KCNQ4* was mapped to chromosome 1p34, a region also hosting *DFNA2*, a locus for autosomal dominant progressive hearing loss (Kubisch et al., 1999). One 13-bp deletion mutation and four missense mutations (G285S, G285C, W276S, and G321S) were identified from families with autosomal dominant progressive hearing loss linked to the *DFNA2* locus (Coucke et al., 1999; Kubisch et al., 1999). The G285S and G285C mutations alter the first glycine residue in the GYG signature sequence of K^+ channel pore. Mutations in these amino acids disrupt the selectivity filter and, in most cases, abolish channel function. An identical change in amino acids at the equivalent position has also been reported in the *KCNQ1* gene of a patient with the dominant LQT1 (Russell et al., 1996). Functional analysis reveals that the mutant channel did not produce current when the cRNA was injected into oocytes, whereas the mutation exerted a dominant-negative effect when coexpressed with wild-type *KCNQ1*. Whereas mutations in *KCNQ1* affect endolymph secretion, the mechanism leading to *KCNQ4*-related hearing loss appears to be in outer hair cells (Kubisch et al., 1999), inner ear, and the central auditory pathway (Kharkovets et al., 2000).

It must be pointed out that in addition to mutations in *KCNQ4*, mutations in *GJB3*, which encodes the connexin 31 component of gap junctions and was mapped to human chromosome 1p33-p35, were identified from the *DFNA2* family with nonsyndromic autosomal dominant hearing loss (Xia et al., 1998). Although at least two or three genes responsible for hearing impairment are located close together on chromosome 1p34, *KCNQ4* mutations may be a relatively frequent cause of autosomal dominant hearing loss.

D. Renal Diseases: Bartter's Syndrome and Kir1.1

Several transporters and ion channels in the renal epithelium play important roles in urine production, fluid balance, and electrolyte metabolism. Genetic analysis reveals that dysfunction of an inward rectifier K^+ channel Kir1.1 is linked to Bartter's syndrome, an autosomal recessive inherited renal tubular disorder characterized by hypokalemia, metabolic alkalosis, hyper-reninism and hyperaldosteronism. Patients have normal or low blood pressure and renal salt loss despite increased plasma renin activity and high serum aldosterone levels (Karolyi et al., 1998; Simon and Lifton, 1998; Scheinman et al., 1999). At least three phenotypically different renal tubulopathies have been identified: antenatal Bartter's syndrome (hyperprostaglandin E syndrome), classic Bartter's syndrome, and Gitelman's syndrome. Of these, polyhydramnios, premature delivery, hypokalemic alkalosis, hypercalciuria, and dehydration at birth characterize the antenatal Bartter's syndrome (hypokalemic alkalosis with hypercalciuria). Children with the antenatal Bartter's syndrome present the typical pattern of impaired salt reabsorption in the thick ascending limb of Henle's loop resulting in the marked ante- and postnatal salt wasting.

Genetic heterogeneity of antenatal Bartter's syndrome has been demonstrated initially by identification of mutations in the *SLC12A1* gene, encoding for the bumetanide-sensitive sodium potassium 2 chloride cotransporter (NKCC2) leading to defective reabsorption of sodium chloride in the thick ascending limb of Henle's loop (Simon et al., 1996a; Vargas-Poussou et al., 1998). Subsequently, several mutations in *KCNJ1*, encoding the apical renal outer medullary inward rectifying K^+ channel (Kir1.1), were identified in patients with antenatal Bartter's syndrome by single strand conformation polymorphism analysis (Simon et al., 1996b; Derst et al., 1997; Feldmann et al., 1998; Vollmer et al., 1998). Functional studies revealed that mutant channels expressed none or significantly reduced currents compared with the wild-type channel. This impaired K^+ flux and loss of tubular K^+ channel function probably prevents apical membrane potassium recycling with secondary inhibition of Na-K-2Cl cotransport in the thick ascending limb of Henle's loop (Derst et al., 1997). The mechanisms underlying impaired Kir1.1 function involve abnormalities in phosphorylation, proteolytic processing, and/or protein trafficking (Schwalbe et al., 1998).

The signs and symptoms of Bartter's syndrome are usually a consequence of hypokalemia. Maintaining normal serum K^+ levels and limiting the degree of metabolic alkalosis are some of the treatment approaches, and potassium supplements and potassium-sparing diuretics are frequently used (Gordon and Stokes, 1994).

E. Metabolic Diseases: Familial Persistent Hyperinsulinemic Hypoglycemia of Infancy and Sulfonylurea Receptor 1

Various types of ion channels are involved in the regulation of electrical activity in the pancreatic β-cell. Of these, the ATP-sensitive K^+ (K_{ATP}) channel plays a critical role in directly linking cellular metabolism to the electrical activity. Opening the ATP-sensitive K^+ channels leads to membrane hyperpolarization and consequently suppression of insulin secretion. Recent genetic analysis has revealed mutations in the ATP-sensitive K^+ channel subunits that may contribute to inappropriate and excessive secretion of insulin.

PHHI is an autosomal recessive disorder characterized by increased irregularity in insulin secretion leading to hypoglycemia, coma, and severe brain damage in children. Both sporadic and familial variants of PHHI are recognized; familial forms are common in communities with high rates of consanguinity where the incidence may be as high as 1 in 2500 live births and is the most common cause of hypoglycemia in newborns

(Aynsley-Green and Hawdon, 1997). Recent genetic linkage analysis has identified mutations in the K_{ATP} channel complex that regulates insulin secretion from pancreatic β-cells. The K_{ATP} channels predominantly determine the resting potential of β-cell and couple cellular metabolism to electrical activity (Ashcroft and Rorsman, 1989; Dukes and Philipson, 1996). When plasma glucose is elevated, increases in intracellular ATP/ADP ratio lead to closure of K_{ATP} channels and membrane depolarization that, in turn, lead to the activation of voltage-dependent Ca^{2+} channel, rise in intracellular Ca^{2+}, and insulin secretion.

The β-cell K_{ATP} channel, like other K_{ATP} channels described in neurons, cardiac, smooth, and skeletal muscle, are inhibited by intracellular ATP, and recent molecular cloning has shown that the channel is an octamer composed of four subunits of the sulfonylurea receptor SUR1 coupled to four subunits of the inward rectifier Kir6.2 (Inagaki et al., 1995a, 1997; Clement et al., 1997; Shyng and Nichols, 1997). Over 28 naturally occurring mutations in SUR1 (Thomas et al., 1995b; Dunne et al., 1997; Verkarre et al., 1998) and two different mutations in Kir6.2 subunits have been identified in families with PHHI (Thomas et al., 1996a; Nestorowicz et al., 1997; Meissner et al., 1999). No K_{ATP} channel activity was observed in β-cells isolated from a homozygous patient or after coexpression of recombinant Kir6.2 and mutant SUR1 (V187D) (Otonkoski et al., 1999). Detailed functional analysis in COS cells by cotransfection of Kir6.2 with various single mutations of SUR1 identified in the PHHI family suggested this lack of K_{ATP} channel activity or reduction of K_{ATP} channel sensitivity to MgADP (Shyng et al., 1998). In fact, patients with mutations in SUR1 either failed to respond to diazoxide or showed diminished sensitivity to treatment (Thornton et al., 1998).

The role of K_{ATP} channels in β-cell function has been evaluated in transgenic mice carrying a dominant-negative form of Kir6.2 (G132S) generated by substituting the glycine lining the pore with serine (Miki et al., 1997). These mice develop hypoglycemia with hyperinsulinemia in neonates and hyperglycemia with hypoinsulinemia and decreased β-cell population in adults. K_{ATP} channel function was found to be impaired in the β-cell of transgenic mice with hyperglycemia. These results imply that the K_{ATP} channel complex might play a significant role in β-cell survival and regulation in insulin secretion, suggesting that modulation of Kir6.2 may offer additional opportunities in treatment of diabetes and related conditions of abnormal glucose regulation. More recently, it has been shown that the SUR1 knockout mice, unlike the Kir6.2 counterpart, are not insulin-hypersensitive, although their β-cells lacks K_{ATP} channels and show spontaneous Ca^{2+} transients similar to those seen in PHHI patients. SUR1 knockout mice were normoglycemic until stressed, unlike in PHHI patients whose glucose levels are persistently low suggestive of a role for K_{ATP}-independent pathways that regulate insulin secretion, at least in mice (Seghers et al., 2000).

III. Disease- and Drug-Induced Regulation of K^+ Channels

A. Cardiac Failure and Hypertrophy

K^+ channels are targets for the actions of transmitters, hormones, or drugs that modulate cardiac functions. Changes in the densities and/or properties of these K^+ channels that occur during the normal development or as a result of damage or disease can have profound physiological consequences (Matsubara et al., 1993; Xu et al., 1996; Yao et al., 1999). Cardiac failure, a pathophysiologic condition with numerous etiologies including myocardial infarction, hypertension, and myocarditis (Wilson, 1997) is characterized by action potential prolongation and, accordingly, altered expression of a variety of depolarizing and hyperpolarizing membrane currents. In an attempt to compensate for the reduction in cardiac function in cardiac failure, the sympathetic nervous system, the renin-angiotensin-aldosterone systems, and other neurohumoral mechanisms are activated. Adaptive changes at the level of the cardiac myocyte include cellular hypertrophy and altered gene expression. Electrical remodeling in cardiac myocytes leading to action potential prolongation is a common finding in human heart failure and in animal models of cardiac hypertrophy. Changes in a wide range of plasma membrane receptors and intracellular signals such as increased intracellular calcium, cAMP, inositol phosphates, and diacylglycerol concentrations are associated with cardiac hypertrophy and failure (Morgan and Baker, 1991; Gopalakrishnan and Triggle, 1990; Wickenden et al., 1998).

A reduction in the current density of the transient outward current (I_{TO}) is the most consistent ionic current change in cardiac hypertrophy and failure (Nabauer and Kaab, 1998; Wickenden et al., 1998; Pinto and Boyden, 1999; Tomaselli and Marban, 1999). This outward repolarizing K^+ current activates and inactivates rapidly with an inactivation constant of ~60 ms (Dixon et al., 1996; Kong et al., 1998). The down-regulation of this current has profound effects on phase 1 and the level of plateau of the action potential, and it also alters currents that are subsequently active along the cardiac action potential. The Kv4.3-containing channel is thought to underlie the bulk of I_{TO} found in the mammalian heart, although Kv1.4 or Kv4.2 channels might represent another fraction of I_{TO} with distinct kinetics in different regions of the heart (Dixon et al., 1996; Kong et al., 1998). By ribonuclease protection assays and whole-cell electrophysiological recording, Kaab et al. (1998) found that the level of Kv4.3 mRNA decreased by 30% in human failing hearts compared with nonfailing controls. This observation correlated with the reduction

in peak I_{TO} density measured in ventricular myocytes isolated from adjacent regions of the heart.

It has been known that action potential durations vary across the myocardial wall and in different regions of the mammalian heart (Litovsky and Antzelevitch, 1989; Fedida and Giles, 1991; Lukas and Antzelevitch, 1993; Di Diego et al., 1996). The density of I_{TO} also varies regionally and transmurally in the heart (Wettwer et al., 1994; Nabauer et al., 1996). Electrophysiological recording from myocytes isolated from patients with aortic stenosis and compensated left ventricular hypertrophy indicates that macroscopic I_{TO} was absent in superficial subendocardial cells, whereas I_{TO} current density was not significantly altered in the deeper layers (Bailly et al., 1997). A region-dependent alteration in the density of I_{TO} current was also observed in the catecholamine-induced hypertrophy in animals (Bryant et al., 1999). It is possible that this region-dependent suppression of I_{TO} current might, in part, underlie the regional heterogeneity in action potential prolongation in cardiac hypertrophy and may predispose to ventricular arrhythmias, a cause of sudden death in patients with cardiac failure.

As discussed later, an approach to the treatment of heart failure would be to normalize K^+ channel gene expression by gene transfer or pharmacologic modulation. Recent studies have shown that thyroid hormone treatment can increase Kv4.2 or Kv4.3 expression at the transcriptional level and enhance the recovery rate from the inactivation of I_{TO} in rat ventricular myocytes (Shimoni et al., 1997; Wickenden et al., 1997). Accordingly, agents with thyroid hormone-like properties might be useful in the treatment of heart failure.

B. Atrial Fibrillation

Atrial fibrillation, the most common arrhythmia in man, is characterized by a marked shortening of the action potential duration, effective refractory period of atria, and a decreased rate of atrial repolarization resulting in increased dispersion of refractoriness as well as changes in atrial conduction velocity (Zipes, 1997; Nattel, 1999). The development of atrial fibrillation can be triggered by rapidly discharging atrial foci (mainly from pulmonary veins) or degeneration of atrial flutter or atrial tachycardia into fibrillation (Chen et al., 1999b; Scheinman, 2000). Risk factors for atrial fibrillation include cardiac diseases such as congestive heart failure, valvular heart disease, and myocardial infarction (Ryder and Benjamin, 1999).

It has been shown that sustained atrial tachycardia causes changes in electrophysiological function to promote the occurrence and maintenance of atrial fibrillation, a process referred to as atrial electrophysiological remodeling (Morillo et al., 1995; Wijffels et al., 1995). Recent studies have revealed that changes in ion channel functions play important roles in atrial electrophysiological remodeling caused by atrial fibrillation. In the canine atrial fibrillation model induced by chronic atrial tachycardia (rapid pacing), isolated atrial myocytes showed significant reductions in L-type Ca^{2+} current and I_{TO} densities, without changes in Kir2.1, hERG, KCNQ1-minK, Ca^{2+}-dependent Cl^- current, or T-type Ca^{2+} currents (Yue et al., 1997). Consistent with this observation, reductions in mRNA levels for Kv4.3, the α_1-subunit of L-type Ca^{2+} channels, and the α-subunit of cardiac Na^+ channels were noted with no changes in mRNA levels for delayed rectifier K^+ channel Kir2.1 or the Na^+/Ca^{2+} exchanger. Western blot analysis further confirmed a reduction in protein expression of Kv4.3 and Na^+ channels, whereas that of the Na^+/Ca^{2+} exchanger was unchanged (Yue et al., 1999; Li et al., 2000). More importantly and consistent with data from the canine atrial fibrillation model, significant reductions in I_{TO} (encoded by Kv4.3) and ultrarapid delayed rectifier (IK_{ur}) (encoded by Kv1.5) as well as L-type Ca^{2+} current densities were observed in atrial myocytes isolated from patients in chronic atrial fibrillation. Furthermore, quantitative Western blot analysis revealed that the expression of Kv1.5 protein was reduced by >50% in both the left and the right atrial appendages of atrial fibrillation (Van Wagoner et al., 1997, 1999). Although abnormalities of K^+ channels may be fundamentally implicated in atrial fibrillation, other factors such as structural changes (Li et al., 1999) or heterogeneous alterations in atrial sympathetic innervation (Jayachandran et al., 2000) may also play critical roles in other forms of atrial fibrillation.

C. Drug-Induced Long-QT Syndromes

Drug-induced precipitation of polymorphic ventricular dysrhythmia, the torsade de pointes, in susceptible individuals by certain H_1 antagonists such as terfenadine has now been linked to the prolongation of the QT interval consequent to inhibition of the IKr channels encoded by the hERG gene (reviewed in Delpón et al., 1999; Taglialatela et al., 2000). These drugs have been shown to block hERG channels in a concentration range similar to that found in the plasma of subjects showing proarrhythmic effects. Similar interactions have been reported for antipsychotics such as sertindole (Rampe et al., 1998), tricyclic antidepressants, and certain antibiotics and anti-emetic agents. Inhibition of another cardiac delayed rectifier, Kv1.5, by H_1 receptor antagonists such as loratadine (Lacerda et al., 1997) and rupatadine (Caballero et al., 1999) has also been suggested to contribute to drug-induced cardiac arrhythmias.

D. Apoptosis and Oncogenesis

K^+ channel activities play important roles in signaling pathways leading to proliferation, differentiation, and cell fusion. Increases in K^+ channel activity and enhanced K^+ efflux are thought to sustain membrane hyperpolarization necessary to facilitate Ca^{2+} entry (Santella, 1998), although additional pathways, such as

control of cellular volume by K^+ channels, might also be involved in cell proliferation (Rouzaire-Dubois and Dubois, 1998; Vaur et al., 1998). A number of studies have suggested membrane hyperpolarization as an essential requirement for cell proliferation. For example, an increase in expression levels of a Ca^{2+}-dependent K^+ channel with strong inward rectification was observed during the G_1 phase of HeLa cells, which progressively declined to a minimum in the S phase and then increased in the M phase (Takahashi et al., 1993). Inhibition of K^+ channels by pharmacological agents has been found to inhibit cell proliferation in normal human lymphocytes (Amigorena et al., 1990; Lin et al., 1993; Rader et al., 1996; Jensen et al., 1999), human melanoma cells (Nilius and Wohlrab, 1992; Lepple-Wienhues et al., 1996), small lung cancer cells (Pancrazio et al., 1993), breast cancer cells (Woodfork et al., 1995), and prostatic cells (Skryma et al., 1997). Changes in expression of an inward rectifying K^+ channel and a noninactivating delayed rectifier K^+ channel are associated with the time course of membrane fusion of myoblast to form multinucleated skeletal muscle fibers (Shin et al., 1997; Occhiodoro et al., 1998). Recently, a gene encoding the human EAG K^+ channel was cloned from myoblasts, localized to chromosome 1q32-41 and shown to be responsible, in part, for changes in membrane hyperpolarization during the myoblast fusion (Occhiodoro et al., 1998).

1. Apoptosis. Apoptosis, or programmed cell death, is a fundamental biological process involved in many physiological and pathological phenomena. This process is predominantly catabolic in nature where cellular macromolecules are broken down by distinct enzymes to be later recycled in healthy cells. Activities of enzymes, nucleases, and caspases that propagate and amplify death signals are K^+-dependent (Bortner et al., 1997; Hughes and Cidlowski, 1999). Recent studies have shown that enhancement of K^+ current is directly involved in apoptosis (Yu et al., 1997, 1999) and oncogenesis (Pardo et al., 1999). In mouse neocortical neurons, a delayed rectifier and tetraethylammonium (TEA)-sensitive K^+ current responsible for neuronal apoptosis was enhanced by serum deprivation or staurosporine. Inhibition of outward K^+ currents with TEA or elevated extracellular K^+, but not with blockers of Ca^{2+}, Cl^-, or other K^+ channels, reduced apoptosis. Exposure to the K^+ ionophore valinomycin or the K_{ATP} channel opener cromakalim induced apoptosis (Yu et al., 1997). Thus, enhanced K^+ efflux through increase in expression of a specific TEA-sensitive and delayed rectifier K^+ channel may mediate certain forms of neuronal apoptosis in disease states. Thymocyte apoptosis induced by dexamethasone, etoposide, γ-irradiation, or ceramide has also been shown to be prevented by the K^+ channel blocker tetrapentylammonium (Dallaporta et al., 1999).

In addition to increased expression of K^+ currents, modulation of K^+ channel function is one of the mechanisms used to induce programmed cell death by a variety of extrinsic and intrinsic signals. For example, the inhibition of Kv1.3 current by tyrosine kinase phosphorylation induced by Fas plays important roles in apoptosis, which is critical to the development of the immune system, and in the elimination of target cells expressing foreign antigens (Szabo et al., 1996). In *Drosophila*, *reaper*, *grim*, or *hid* gene expression triggers apoptosis in a caspase-dependent manner. The peptides encoded by these genes share a common feature in that their N termini are similar to those of the *Shaker* K^+ channel that block channel and lead to fast inactivation. Mutations that reduce the apoptotic activity of *reaper* also reduced the peptide's ability to induce channel inactivation. Thus, blocking a *Shaker* K^+ channel by peptides encoded by *reaper*, *grim*, or *hid* gene was suggested to be involved in apoptosis (Avdonin et al., 1998).

2. Oncogenesis. Modulation of K^+ channels is involved in Ras/Raf signal transduction in oncogenic transformation (Collin et al., 1990; Yatani et al., 1991; Huang and Rane, 1994; Decker et al., 1998). Recent studies have shown a high level of an intermediate conductance Ca^{2+}-activated K^+ current (IK_{Ca}) in Ras-transformed fibroblasts but not in the untransformed counterparts (Rane, 1991). High levels of expression of IK_{Ca} have also been observed in rat prostate cancer cell lines, AT2.1 and MatLyLyu, suggesting hyperactivity of the Ras/MAPK pathway in prostatic cancer and that IK_{Ca} plays important roles in regulating cell growth (Rane, 2000). Similarly, the hERG was shown to be sequentially expressed during neuronal development and to participate in the regulation of membrane potential in mammalian neuroblastoma cells (Arcangeli et al., 1995, 1997). The hERG, and the related *ether-a-go-go* K^+ channels are expressed in a variety of tumor cell lines (Bianchi et al., 1998; Pardo et al., 1999), the inhibition of which causes a significant reduction of cell proliferation. Moreover, the expression of rEAG favors tumor progression when transfected cells are injected into immunosuppressed mice, and overexpression of rEAG K^+ channels in Chinese hamster ovary or NIH 3T3 cells induces significant features characteristic of malignant transformation (Pardo et al., 1999). Taken together, these studies suggest that these K^+ channels play crucial roles in oncogenesis.

E. Alzheimer's Disease

Alzheimer's disease is the most prevalent cause of progressive declining cognitive function, loss of memory, and late stage decreasing physical deterioration in the elderly. It is characterized pathologically by the presence of intracellular neurofibrillary tangles and extracellular neuritic plaques consisting of deposits of the β-amyloid ($A\beta$), a 39- to 43-amino acid peptide proteolytically derived from β-amyloid protein precursor (β-APP). In Alzheimer's disease, significant neuronal cell death is found in the temporal and parietal cortex,

hippocampus, amygdala, and basal forebrain cholinergic system. Several mechanisms have been linked to progressive neurodegenerative disorder, such as alterations in amyloid precursor protein metabolism, cholinergic transmission, calcium homeostasis, oxidative metabolism, and protein kinase C transduction systems (Mattson et al., 1993; Hensley et al., 1994; Ito et al., 1994; Yankner, 1996; Yu et al., 1998). As discussed below, dysfunction of K^+ channels in both central nervous systems and peripheral tissues has been reported. It is plausible, however, that any association of K^+ channel defects with the pathophysiology of Alzheimer's disease may be indirect or secondary in nature consequent to generalized degeneration associated with the disease.

1. β-Amyloid. K^+ channel dysfunction in Alzheimer's disease was initially suggested by radioligand binding studies using apamin, the bee venom octadecapeptide that blocks small conductance Ca^{2+}-activated K^+ channels responsible for afterhyperpolarization of neurons (Ikeda et al., 1991). In hippocampus, a reduction of ^{125}I-apamin binding sites in the subiculum and CA1 regions was found in patients with Alzheimer's disease. The reduction of ^{125}I-apamin binding sites in the subiculum correlated with cell density but not neuritic plaque density, indicating discrete loss of small conductance of Ca^{2+}-activated K^+ channels within the hippocampal formation. In hippocampal neurons from neonatal rats, Aβ was shown to inhibit voltage-dependent fast-inactivating K^+ currents (Good et al., 1996). This inhibition results in abnormally large increases in intracellular Ca^{2+} levels upon depolarization of the neuron leading to neurotoxicity (Good and Murphy, 1996).

Other evidence linking Aβ-induced abnormal K^+ to the neuronal cell death was revealed by in vitro studies using a cholinergic septal cell line, SN56 (Colom et al., 1998). These cells exhibited a tetraethylammonium-sensitive outward K^+ current with delayed rectifier characteristics. Addition of Aβ increased K^+ current density some 44 to 66% and decreased cell viability by 25 to 39%. TEA (10 to 20 mM) or K^+ depolarization inhibited outward currents, widened action potentials, elevated $[Ca^{2+}]_i$, and inhibited more than 68% of the Aβ-induced toxicity. These data suggest that a K^+ channel with delayed rectifier characteristics may play an important role in Aβ-mediated toxicity in this septal cholinergic cell line (Colom et al., 1998).

In peripheral tissues, K^+ channel dysfunction was initially identified in fibroblasts from patients with Alzheimer's disease where a 113-pS TEA-sensitive K^+ channel was absent compared with normal human fibroblasts (Etcheberrigaray et al., 1993). This defect was mimicked in normal fibroblasts by the addition of β-amyloid protein (Etcheberrigaray et al., 1994). TEA depolarized and elevated intracellular Ca^{2+} levels in young and aged control fibroblasts but not in fibroblasts from Alzheimer's disease patients, supporting the dysfunction of TEA-sensitive K^+ channels in the disease. Rb^+ flux through apamin and charybdotoxin-sensitive Ca^{2+}-activated K^+ channels was selectively impaired in fresh, noncultured platelets from patients with Alzheimer's-type dementia, although the α-dendrotoxin-sensitive voltage-dependent K^+ channel was not affected compared with nondemented controls (de Silva et al., 1998). β-Amyloid protein also enhanced phytohemagglutinin-induced Ca^{2+} rise in T-lymphocytes, consistent with the hypothesis that enhanced calcium responses serve as a general feature of β-amyloid neurotoxicity (Eckert et al., 1993). However, patch-clamp analysis indicated that T-lymphocyte K^+ channels are not functionally deficient in Alzheimer's disease, and that β-amyloid protein does not mediate an alteration of their currents (Cohen et al., 1996), suggesting Aβ might induce toxicity through alternative pathways.

2. β-Amyloid Protein Precursor. β-APP, the source of the fibrillogenic Aβ, is a membrane-spanning and multifunctional protein that is widely expressed in the nervous system. β-APP is axonally transported and accumulates in presynaptic terminals and growth cones. A secreted form of β-APP (sAPP) is released from neurons in response to electrical activity and plays important roles in learning, memory, and cell survival (Roch et al., 1994; Mattson, 1997; Meziane et al., 1998; Dodart et al., 2000). In addition to Aβ-induced neurotoxicity via potential modulation of K^+ channel function, a study revealed that sAPP can suppress action potential and hyperpolarize hippocampal neurons by activating large conductance Ca^{2+}-activated K^+ channels leading to suppression of intracellular Ca^{2+} concentration (Furukawa et al., 1996). These results suggest that the effects of β-APP on synaptogenesis and synaptic plasticity might, in part, mediate through activation of Ca^{2+}-activated K^+ channels and that the abnormalities in β-APP processing or sAPP might contribute to the neurodegenerative process in Alzheimer's disease.

3. Presenilins. The presenilins are proteins that contain multiple transmembrane domains and localize primarily to the endoplasmic reticulum and Golgi apparatus. Although the precise functions of presenilins are not totally understood, presenilins are involved in the proteolytic processing of β-amyloid precursor proteins and play important roles in the notch signaling during embryonic development and/or cellular differentiation (Kim and Tanzi, 1997; Chan and Jan 1999; Haass and De Strooper, 1999; Czech et al., 2000). Genetic linkage analysis showed that mutations in presenilin 1 (PS-1, mapped on chromosome 14) and presenilin 2 genes (PS-2 on chromosome 1) yielding abnormal release of amyloidogenic peptide from amyloid precursor protein have been linked to the autosomal dominant early onset of familial Alzheimer's disease (Clark et al., 1995; Rogaev et al., 1995; Schellenberg, 1995; Sherrington et al., 1995). Based on the multiple membrane-spanning topology, it was proposed that presenilins might function as, or as part of, a channel, transporter, or pore (Li and

Greenwald, 1996). Using in vitro expression in HEK-293 cells, a recent study has revealed that expression of wild-type PS-1 or PS-2 increases outward K^+ current densities. In HEK-293 cells transiently transfected with PS-1 (S290C) or PS-1 (G209V), two missense mutations associated with early onset Alzheimer's disease, mean outward K^+ current densities are also shown to be increased in HEK-293 cells expressing the S290C mutant but not with the G209V mutant. Expression of wild-type PS-1 in neonatal rat ventricular myocytes also results in increased outward K^+ currents, whereas no detectable effects on membrane currents were seen in COS-7 cells transfected with PS-1. These results suggest that the presenilins do not actually form K^+ channels, but rather that these proteins up-regulate functional K^+ channel expression (Malin et al., 1998). Thus, presenilins could regulate neuronal K^+ channel expression, and mutations in PS-1 or PS-2 can, in part, result in profound changes in neuronal excitability, which may contribute to the cognitive decline commonly associated with Alzheimer's disease to some extent.

F. Neuromuscular Disorders

Mutations in a variety of ion channels, including Na^+, Ca^{2+}, and Cl^- channels, have been found to underlie various forms of human neuromuscular disorders. The defects of ion channels lead to the aberrant excitability of muscle fibers that gives rise to periodic paralysis or myotonia (for reviews see Cannon, 1996; Engel et al., 1998). In addition to inherited genetic diseases, diverse neuromuscular disorders are attributed to antibody-mediated autoimmunity where the extracellular domains of receptors or ion channels are the primary targets of autoantibodies. For example, myasthenia gravis is caused by autoantibodies to nicotinic acetylcholine receptors at the neuromuscular junction, which cause weakness of the skeletal muscle (Richman and Agius, 1994). The autoantibodies that interfere with neurotransmitter release by binding to presynaptic voltage-dependent Ca^{2+} channels underlie the Lambert-Eaton myasthenic syndrome, which is often found in patients with small cell lung cancer (Kim and Neher, 1988; Pelucchi et al., 1993). In acquired neuromyotonia (Isaacs' syndrome), where hyperexcitability of peripheral motor nerves leads to muscle twitching during rest, cramps during muscle contraction, impaired muscle relaxation, and muscle weakness, autobodies directed against 4-aminopyridine or α-dendrotoxin-sensitive K^+ channels in motor and sensory neurons were detected (Shillito et al., 1995; Hart et al., 1997). These antibodies mainly suppress voltage-gated K^+ channels (Kv1.1 and Kv1.6) with no change in gating kinetics and lead to peripheral nerve hyperexcitability (Nagado et al., 1999). In humans with hypokalemic periodic paralysis caused by mutations of the 1,4-dihydropyridine receptor of the voltage-gated calcium channel, diminished skeletal muscle K_{ATP} channel activity has also been reported (Tricarico et al., 1999).

IV. Pharmacological Considerations

As discussed in the preceding sections, several genetically linked and acquired diseases involve alterations in the function of K^+ channels. Genetic linkage studies have been pivotal in elucidating the role of many K^+ channels in pathophysiologic and physiologic conditions. More importantly, these findings provide a basis to develop appropriate therapy for various diseases. Continuing pharmaceutical interest revolves around the discovery and development of selective organic modulators of various classes of K^+ channels (Colatsky, 1998; Curran, 1998; Kaczorowski and Garcia, 1999). Enthusiasm in the K^+ channel arena is driven by the realization that class III antiarrhythmic agents and antidiabetic sulfonylureas act as antagonists at specific K^+ channel classes and that a variety of K^+ channel inhibitors and openers offer significant therapeutic opportunities in areas ranging from cardiac, vascular, and nonvascular muscle, neuronal, immune, and secretory systems to modulation of hair follicle growth (Table 3). Gene delivery and selective targeting of channel proteins by antisense oligonucleotides represent emerging approaches. With advances in molecular biology and antisense technology, therapeutics based on gene delivery, with precise control of the level and distribution of ion channel expression into mammalian neuronal, cardiac, hair cells, and other cell types, are currently being investigated (Holt et al., 1999; Johns et al., 1999; Hoppe et al., 2000).

However, key hurdles in targeting K^+ channels remain to be resolved. Given the diversity of K^+ channel subunits and the potential to vary the constituents to form heteromeric channel complexes to alter expression, cellular targeting, and biophysical and pharmacological properties in native cell types, it is difficult to know the precise composition of channel complexes in vivo. The latter, together with information on tissue-specific localization and the availability of high-throughput in vitro assays predictive of in vivo drug activity and selectivity, is seldom available. This is an important issue, which has not been addressed to the full extent, as efforts are launched to design openers and/or blockers of various classes of potassium channel modulators. Nevertheless, over the past decade or so, intense medicinal chemistry efforts have focused on the synthesis and development of modulators of various voltage-gated K^+ channels, calcium-activated K^+ channels and ATP-sensitive K^+ channels (Figs. 3 and 4; Tables 3 and 4).

A. Voltage-Gated K^+ Channels

1. Kv1.3 Channels. The Kv1.3 channels, members of the voltage-gated K^+ channel family expressed predominantly in human lymphocytes, have been widely exploited as pharmacological targets for immunosuppressive

TABLE 3
Potassium channel openers

Channel Family	Therapeutic Indication(s)	Compounds
KCNQ2/KCNQ3	Epilepsy	Retigabine (also $GABA_A$ agonist)
BK_{Ca}	Cerebral ischemia	BMS-204352
		NS 004 (also inhibits other K^+ channels)
	Coronary disorders	NS 1608, NS1619 (also Ca^{2+} channel inhibitor)
	Antipsychotic	
	Urinary incontinence	NS8
	Pollakisuria	
K_{ATP}	Hypertension,	Pinacidil
	ischemic heart disease,	Diazoxide
	heart failure,	Nicorandil
	Asthma	Aprikalim (RP 52891)
		Bimakalim (EMD52692)
		Celikalim
		Cromakalim
		Emakalim
		NIP121
		RO 316930
		RWJ 29009
		SDZ PCO 400
		Rimakalim (HOE234)
		Symakalim (EMD 57283)
		YM-099, YM-934
	Myocardial ischemia	BMS180448
		U 89232 (BMS 189365)
		(Mito K_{ATP}?)
	Alopecia	P1075, minoxidil
	Urinary incontinence	ZM244085, ZD6169, WAY133537, WAY151616, ZD0947
	Erectile dysfunction	PNU83757

therapy. Selective blockers of these channels depolarize membrane to attenuate calcium influx and inhibition of T cell activation in vitro and immunosuppression in vivo (Cahalan and Chandy, 1997). Many peptides isolated from scorpion venoms and sea anemone potently block Kv1.3 channels and inhibit T-lymphocyte activation. Inhibition of these channels by margatoxin was initially shown to prevent T cell activation and attenuate immune responses in vivo (Koo et al., 1997). Several nonpeptide analogs, such as dihydroquinolines, WIN 17317-3 (Hill et al., 1995) and CP-339,818 (Nguyen et al., 1996), piperidines, UK 78,282, (Hanson et al., 1999), and certain alkoxypsoralenes (Wulff et al., 1998) have been shown to block Kv1.3 channels and/or inhibit human T cell activation in vitro. Despite this in vitro evidence, there has been little in vivo demonstration until recently that blockade of Kv1.3 will attenuate immune responses, the latter possibly due to species differences, since in many rodent peripheral T cells these channels do not appear to set membrane potential. However, these channels appear to be present on peripheral T cells of minipigs, and Koo et al. (1999) have shown that the nortriterpene, correolide, and its analogs extracted from the tree *Spachea correae* can block Kv1.3 channels and inhibit delayed-type hypersensitivity response to tuberculin in minipigs (Koo et al., 1999). The Kv1.3 modulators described thus far could serve as tools for the further design of immunosuppressive agents because many of these compounds lack desirable potencies, selectivity, and pharmacokinetic profile. For example, a study with radiolabeled WIN 17317-3 has shown that this compound is also a potent blocker of brain type IIa sodium channels (Wanner et al., 1999).

2. Cardiac Delayed Rectifier K^+ Channels. The goal of developing a class III antiarrhythmic agent effective against ventricular arrhythmias while reducing hemodynamic liabilities remains to be realized, but should now be accelerated with the understanding of the molecular components of cardiac delayed rectifiers, i.e., IKs (KvLQT1-minK), IKr (hERG), and IK_{ur} (Kv1.5) channels. The currently available class III drugs amiodarone (Kodama et al., 1999) and sotalol (Anderson and Prystowsky, 1999) possess properties beyond the realm of a pure class III effect (Roden, 1993; Nair and Grant, 1997; Sager, 1999).

Novel antiarrhythmic drugs belonging to the class III type have now become available that block a specific ionic current (e.g., dofetilide that blocks IKr) or block multiple ionic channels (e.g., ibutilide and azimilide) to prolong atrial and ventricular action potentials without unwanted pharmacological effects. Since IKr blockers increase action potential duration and refractoriness both in atria and ventricle without affecting conduction per se, theoretically they represent potentially useful agents for the treatment of arrhythmias, although they may have an enhanced risk of proarrhythmia at slow heart rates (Table 4).

As noted previously, cardiac tissues express rapidly activating delayed rectifier currents, designated IK_{ur}, in contrast to the classical IKr and IKs channels. The Kv1.5 subunit is the major component of the cardiac ultrarapid delayed rectifier in human atria as revealed by localization (Mays et al., 1995) and antisense oligonucleotide studies in cultured adult human atrial myocytes (Feng et al., 1997). Association with Kvβ1.2

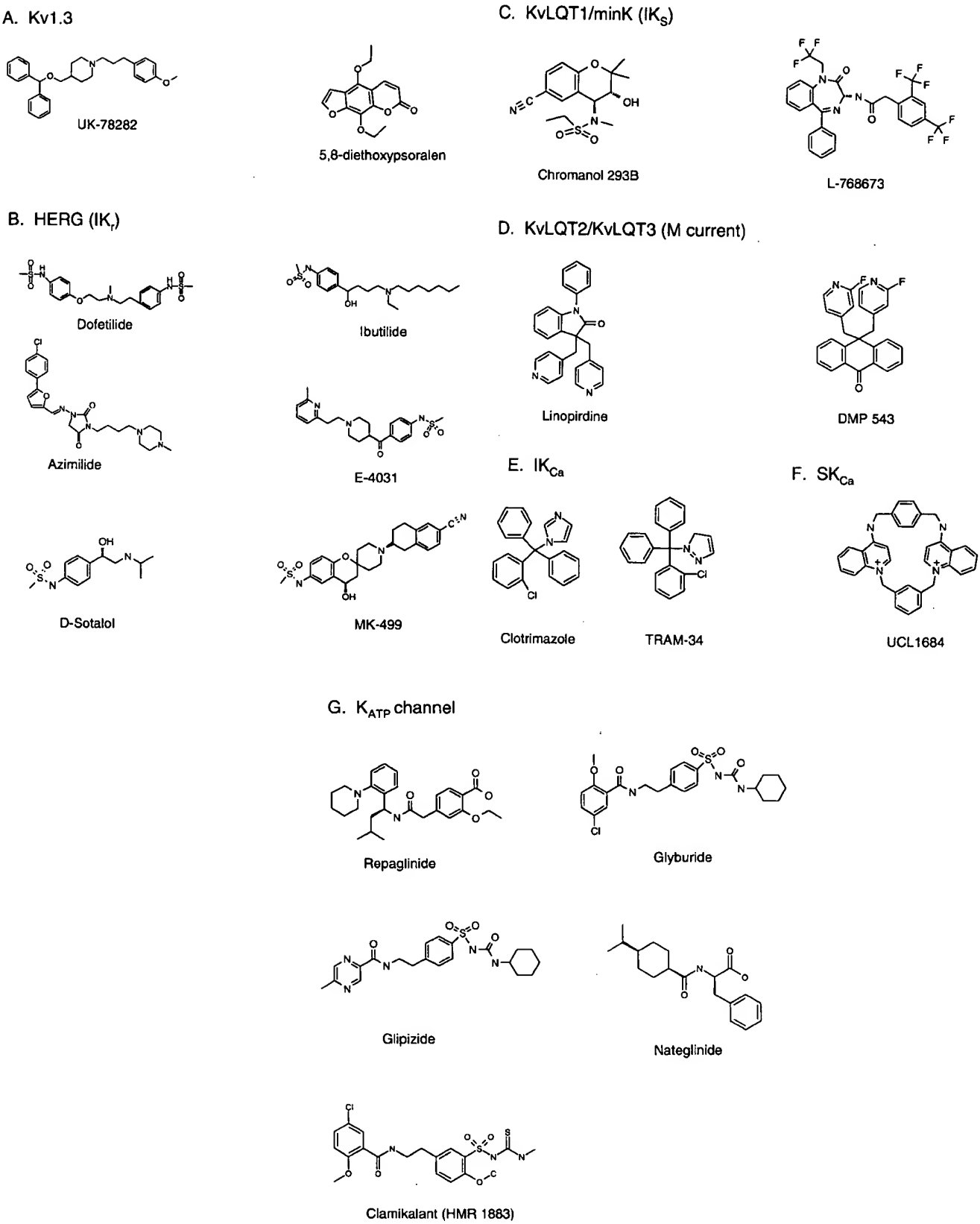

FIG. 3. K+ channel blockers. Shown are blockers of voltage-gated, calcium-activated, and ATP-sensitive K+ channels.

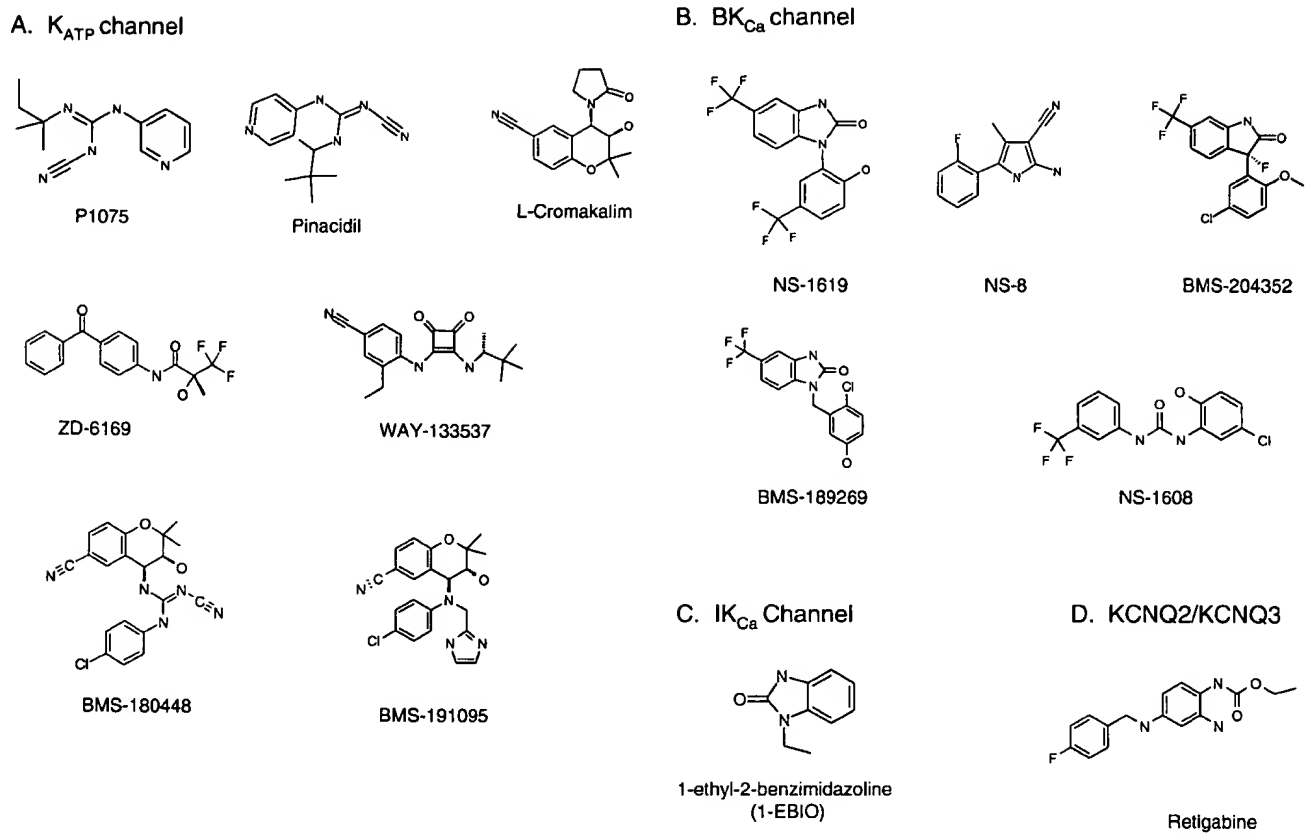

FIG. 4. K^+ channel openers. Shown are openers of voltage-gated, calcium-activated, and ATP-sensitive K^+ channels.

TABLE 4
Potassium channel blockers

Channel Family	Therapeutic Indication(s)	Compounds
Kv1.3	Immunosuppressant	CP308408, UK 78,282
Kv1.5	Atrial fibrillation	
Kv (other)	Multiple sclerosis (axonal regeneration)	Fampridine (4-aminopyridine)
	Epilepsy, ischemia	BIIA 0388
hERG/IKr	Atrial fibrillation/flutter	Dofetilide (Tikosyn)
	Arrhythmia	Ibutilide (Corvert injection) (also increases Na^+ current)
		Almokalant
		E4031
		MK 499
		Sematilide
		D-Sotalol
KvLQT1-minK/IK_S	Arrhythmia	Chromanol 293B
		HMR1556
		E-047/1
		L768673
IKr and IKs	Arrhythmia, angina	Ambasilide (LU 47710)
		Azimilide (NE 10064) (also L-type CaCh blocker)
		Tedisamil (also Na^+ channel blocker)
I_{TO}	Arrhythmia	Clofilium
KCNQ3/KCNQ4	Alzheimer's disease	DMP543
K_{ATP}	Ventricular arrhythmia, heart failure, cardiac arrest	HMR 1098, HMR 1883
	Type II diabetes	Tolbutamide
		Chlorpropamide
		Glibenclamide
		Glipizide
		Nateglinide
		Repaglinide

subunits can also alter functional properties of Kv1.5 channels (Majumder et al., 1995). Selective blockers of Kv1.5 channels could be potentially beneficial for the treatment of cardiac arrhythmias because such agents could retard repolarization and prolong refractoriness selectively in cardiac myocytes (Nattel et al., 1999).

Gene transfer of delayed rectifier K^+ channels represents an emerging strategy for the control of arrhythmias

triggered by altered cardiac repolarization. Myocytes isolated from adult rabbit ventricular myocytes in culture which demonstrate longer action potentials and frequent early-after depolarizations when maintained in culture, were reversed following adenoviral gene transfer of the hERG gene (Nuss et al., 1999). Infection with a recombinant adenovirus containing the hERG gene selectively enhanced the E-4031-sensitive currents without affecting the density of transient outward currents, suppressed early-after depolarizations, and lengthened the refractory period. Action potentials from failing dog hearts were also reversed after exposure to an adenovirus that overexpresses *Shaker* K^+ channels (Nuss et al., 1996). Further refinement of techniques to effectively control the level and to ensure homogenous distribution of transgene expression at the target organ is likely to be forthcoming (Hoppe et al., 2000).

As noted previously, it has also become increasingly important to avoid interactions of many noncardiovascular medicinal products with cardiac ion channels (reviewed in Pourrias et al., 1999). Certain H_1 antagonists, such as astemizole and terfenadine, and the prokinetic agent cisapride are capable of prolonging the QT interval and inducing torsade de pointes in susceptible individuals through inhibition of IKr channels encoded by hERG gene. Similar interactions have also been reported for certain antipsychotics such as sertindole (Rampe et al., 1998), tricyclic antidepressants, and some antibiotics. Inhibition of Kv1.5 channels by H_1 receptor antagonists such as loratadine (Lacerda et al., 1997) and rupatadine, a dual antagonist of H_1, and platelet-activating factor receptors (Caballero et al., 1999) has also been suggested to contribute to drug-induced cardiac arrhythmias. Prolongation of cardiac repolarization reported with the $5HT_3$ receptor antagonist, ondanestron, has been attributed to inhibition, albeit only 30%, of hERG channels (Kuryshev et al., 2000a). Needless to note, these pharmacologic misfortunes underscore the importance of evaluation of potential inhibition of these cardiac channels during the early developmental phase of novel compounds because drugs with minimal or no potential to block hERG or Kv1.5 channels are likely to possess cardiac safety advantages.

3. KCNQ2/KCNQ3 Channels. Unlike *KCNQ1, KCNQ2* and *KCNQ3* are present exclusively in the nervous system and coassemble to form heteromultimers that underlie the M-current (Wang et al., 1998) critical to neuronal excitability in the nervous system (Brown, 1988). The potential for targeting the KCNQ2/KCNQ3 combination as a drug target is underscored by the findings that compounds such as linopirdine [DuP 996, 3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one] and XE991 developed as cognition enhancers are blockers of cloned KCNQ channels (Lamas et al., 1997; Eid and Rose, 1999). Linopirdine, a putative cognition enhancing drug, increases acetylcholine release in rat brain tissue and improves performance in animal models of learning and memory (Schnee and Brown, 1998). Although clinical data with linopirdine were largely inconclusive, analogs such as XE991 and DMP543 with superior pharmacological and pharmacodynamic properties have entered development as orally active acetylcholine-releasing agents with potential in Alzheimer's disease (Zaczek et al., 1998). The KCNQ1/minK complex was 14- to 18-fold less sensitive to XE991 blockade compared with either KCNQ1 alone or neuronal KCNQ2/KCNQ3 combination, revealing a much desired degree of selectivity for this compound for neurotransmitter release over cardiac function (Wang et al., 2000). More recently, retigabine (D-23129), reportedly in phase II clinical studies for the treatment of epilepsy, has been shown to activate KCNQ2/KCNQ3 channels expressed in Chinese hamster ovary cells in a partially linopirdine-sensitive manner, suggesting that M-channel activation may be a novel mode of action for anticonvulsant drugs (Main et al., 2000; Rundfeldt and Netzer, 2000).

B. Calcium-Activated K^+ Channels

The recent molecular cloning of various calcium-activated K^+ channels has renewed enthusiasm for the development of modulators for these channels. These channels, critically dependent on intracellular calcium for channel opening, were initially differentiated largely on the basis of biophysical (conductance, voltage dependence) and differential toxin sensitivity into large, intermediate, and small conductance Ca^{2+}-activated K^+ channels. Distinct genes are now known to encode the three subfamilies of calcium-activated K^+ channels, i.e., large conductance (BK_{Ca}) (α-subunit and its splice variants), small conductance (Sk1, Sk2, and Sk3), and intermediate conductance channels (reviewed in Vergara et al., 1998; Castle, 1999; Wallner et al., 1999b). The search for organic modulators of various Ca^{2+}-activated K^+ channels with the potential to be developed as therapeutic agents has been actively explored by functional screening using many of the recombinant channels (Kaczorowski and Garcia, 1999).

1. Large Conductance Channels. The BK_{Ca} α-subunit cloned from either *Drosophila* (*Slo*) or mammalian (*mSlo, hSlo*), in combination with different β-subunits, β1, and more recently β2 to β4, now extends diversity of BK_{Ca} channels. Initial modulators reported include activators such as glycosylated triterpenes (dehydrosoyasaponin-I) and several indole diterpene blockers, such as paxilline, verruculogen, penitrem A, and aflatrem (Kaczorowski et al., 1996). Activators of BK_{Ca} channels include the benzimidazolones, such as NS-1619 and NS-004. However, these compounds are, in general, not very potent or highly selective. More recently, openers of BK_{Ca} channels have been developed as neuroprotective agents. One such compound, BMS-204352, is in advanced trials as a stroke neuroprotectant (Hewawasam et al., 2000). NS-8, a pyrrole derivative shown to activate BK_{Ca} channels, is under investigation for the treatment of urinary incontinence (Tanaka et al., 1998). The potential for BK_{Ca} modulators in

the treatment of erectile dysfunction has been underscored by recent studies with the BK_{Ca} channel α-subunit (Christ et al., 1998). Intracavernous injection of *hSlo* DNA was capable of altering nerve-stimulated penile erection and was associated with a significant elevation in intracavernous pressure at least until two months postinjection. Interestingly, the expression of the *hSlo* message was highest in the corpus cavernosum tissue and minimal in other tissues examined, raising the possibility that such localized delivery of K^+ channel genes may provide another avenue for achieving end organ selectivity.

2. Intermediate Conductance Channels. Blockers of the IK_{Ca} channel have long been proposed for therapy in sickle cell anemia, diarrhea, and rheumatoid arthritis; clotrimazole, an inhibitor of the IK_{Ca} channel in red blood cells, has been used for this purpose (Brugnara et al., 1995; de Franceschi et al., 1996). However, the inhibition of cytochrome P450 enzyme by clotrimazole limits its therapeutic applications. Recently, a more selective and potent inhibitor of IK_{Ca} channel, TRAM-34 (1-[(2-chlorophenyl)diphenylmethyl]-1*H*-pyrazole), with no effect on cytochrome P450 activity, has been reported (Fig. 3) (Wulff et al., 2000). Although not highly specific, 1-ethyl-2-benzimidazolinone (1-EBIO) and the clinically used benzoxazoles, chlorzoxazone and zoxazolamine, are described as pharmacological activators of the IK_{Ca} channel (Syme et al., 2000). Inhibitors of IK_{Ca} may also be useful as immunosuppressive agents because these channels are up-regulated following antigenic or mitogenic stimulation (Khanna et al., 1999). IK_{Ca} channels may also serve as an effector for mitogenic Ras/MAPK signaling in fibroblasts and other cell types, including prostate cancer cells (Rane, 2000). Openers of IK_{Ca} channels may be therapeutically beneficial in cystic fibrosis and peripheral vascular disease, as well (Edwards, 1998).

3. Small Conductance Channels. The SK_{Ca} channel, first identified in cultured rat skeletal muscle, was shown to be the receptor inhibited by the bee venom peptide apamin (Blatz and Magleby, 1986). Activation of apamin-sensitive SK_{Ca} channels underlies a component of the after hyperpolarization current in neurons that parallels the rise and fall of intracellular calcium levels (Sah and Clements, 1999). Besides apamin, other blockers of SK_{Ca} channels, albeit less selective, include tubocurarine and dequalinium. Many dequalinium analogs with varying potencies and selectivities for blocking IK_{Ca} and SK_{Ca} channels have been described (Malik-Hall et al., 2000). For example, the bisaminoquinolium cyclophane UCL 1684 is about 5000-fold more selective in inhibiting SK_{Ca} channels compared with IK_{Ca}-type channels. Recently, conditional overexpression of a small conductance K^+ channel, Sk3, induced abnormal breathing patterns during hypoxia and compromised parturition in mice by changes in uterine smooth muscle function (Bond et al., 2000). The availability of selective SK_{Ca} modulators will permit evaluation of their potential role in epilepsy, sleep apnea, neurodegenerative, and smooth muscle disorders.

C. ATP-Sensitive K^+ Channels

K_{ATP} channels, a family of weak inward rectifiers inhibited by intracellular ATP that couple cellular energy metabolism to membrane electrical activity, have perhaps been the most widely explored K^+ channels in terms of therapeutic potential (Noma, 1983; Ashcroft and Ashcroft, 1990; Gopalakrishnan et al., 1993). First generation K^+ channel openers (KCOs), including cromakalim and pinacidil, have been known to activate glyburide-sensitive K_{ATP} channels in a variety of vascular and nonvascular tissues (Edwards and Weston, 1993). A variety of structurally diverse KCOs, including benzopyran (cromakalim), cyanoguanidines (pinacidil), and nitroethylene analogs, have been evaluated as potential antihypertensive agents during the past 15 years, although only nicorandil, and to a lesser extent diazoxide, have been used in cardiovascular medicine, in part due to the availability of other classes of agents for these indications.

The recent cloning and expression of K_{ATP} channel components has provided insight into the observed heterogeneity in the pharmacologic profile of KCOs (reviewed in Aguilar-Bryan et al., 1998). As noted previously, the K_{ATP} channel expressed in pancreatic β-cells is a multimeric complex composed of Kir6.2 and the sulfonylurea receptor SUR1 (Clement et al., 1997; Lorenz et al., 1998). From expression studies using rat or mouse SUR subunits, it is thought that the molecular composition of the cardiac/skeletal muscle channel is SUR2A/Kir6.2, whereas SUR2B is thought to be one of the subunits constituting the smooth muscle type K_{ATP} channels. More recently, SUR2 splice variants that lack either exon 14 or exon 17 have been identified by RNA analysis (Chutkow et al., 1999; Davis-Taber et al., 2000). With the emerging diversity of K_{ATP} channel combinations, it could be anticipated that tissues may contain a predominance of certain isoforms involved in various functions ranging from transmitter release to ischemic protection and may be selectively targeted for development of tissue-selective compounds for the treatment of several cardiac and smooth muscle disorders.

Recent efforts have focused on the development of second generation openers of K_{ATP} channels for nonvascular indications including bladder overactivity, irritative bowel syndrome, airway hyper-reactivity, erectile dysfunction, and as cardioprotective agents for the ischemic myocardium (Morley, 1994; Garlid et al., 1997). Compounds investigated for the treatment of bladder overactivity such as ZM-244085, ZD-6169, or WAY-133537 have been shown to activate K_{ATP} channels, relax bladder smooth muscle, and exhibit modest in vivo selectivity (Howe et al., 1995; Wojdan et al., 1999; Gopalakrishnan et al., 1999). Analogs derived from the benzopyran nucleus, including BMS-180448 and BMS-191095, display selectivity for

cardioprotective over vasorelaxant effects relative to the nonselective KCO, cromakalim. BMS-180448 has been shown to have cardioprotective effects at concentrations that do not affect action potential shortening, indicative of activation of a K_{ATP} channel other than the plasma membrane K_{ATP} channel. The cardioprotective effects of the antianginal drug nicorandil have been shown to be via activation of mitochondrial K_{ATP} channels (Sato et al., 2000). Mammalian cells transfected with K_{ATP} channel subunits Kir6.2 and SUR1 showed resistance to hypoxia reoxygenation, and a therapeutic approach based on gene delivery of K_{ATP} subunits in tissues vulnerable to hypoxia reoxygenation and damage has also been suggested (Jovanovic et al., 1998a,b). KCOs examined for airway hyperreactivity include SDZ 217–744, with reported improved selectivity of inhibition of airway hyperactivity relative to cromakalim (Williams et al., 1990). K_{ATP} channel openers have also been investigated for the potential treatment of male erectile dysfunction. Pinacidil, cromakalim, and nicorandil or its analogs have shown increases in intracavernosal pressure by relaxing corporal smooth muscle, which leads to initiation and maintenance of erection (Moon et al., 1999; Vick et al., 2000), providing proof of principle that such compounds, if delivered directly into the corpus smooth muscle, could be a viable treatment option. The basis for the reported modest in vivo selectivity of second generation KCOs could, in principle, arise from interactions with distinct K_{ATP} channel combinations or, more plausibly, from physiologic or pharmacokinetic factors. For instance, studies aimed at elucidating the basis for the cardioprotective effect of KCOs reveal a role for the mitochondrial K_{ATP} channel, the molecular composition of which appears to be somewhat distinct from sarcolemmal K_{ATP} channels (Garlid et al., 1997; Szewczyk and Marban, 1999).

Sulfonylureas such as glibenclamide and glipizide that block K_{ATP} channels in pancreatic β-cells have been used for the treatment of type II diabetes for over 30 years, and newer agents with diminished propensity for sustained hypoglycemic potential continue to be developed. More recently, it has been demonstrated that transfection of SUR1 and Kir6.2 into an insulin-secreting cell line (NES 2Y β-cells) from PHHI patients can restore glucose-dependent insulin release. This opens up the potential for gene therapy to alleviate β-cell dysfunction in PHHI and diabetes (Dunne et al., 1997; Macfarlane et al., 2000). Blockers of K_{ATP} channels such as PNU-37883A have also been evaluated as diuretics or as antiarrhythmic agents (Humphrey and Ludens, 1998). More recent focus continues in the identification of cardioselective K_{ATP} channel blockers for the prevention of ischemia-induced ventricular fibrillation. This has been underscored by the notion that during acute myocardial infarction, activation of ATP-sensitive K^+ currents results in action potential duration shortening and elevation of interstitial $[K^+]$ accumulation that may contribute to reentry arrhythmias and cardiac death (Gögelein et al., 1998). HMR 1883, a relatively cardioselective K_{ATP} channel blocker with modest selectivity for cardiac K_{ATP} over the pancreatic K_{ATP}, prevented ventricular fibrillation in dogs at doses that did not affect plasma insulin or blood glucose. Such compounds may prove useful in the treatment of ventricular arrhythmias without pancreatic side effects or the liabilities of nonselective blockers under ischemic conditions.

D. Two-Pore K^+ Channels

The more recently identified two-pore K^+ channels, including TWIK, TREK, TASK, and TRAAK genes (Table 1), thought to function as background channels involved in the modulation of resting membrane potential in various cell types could emerge as attractive targets for discovering novel neuroprotective and anesthetic agents (Lesage and Lazdunski, 1999). The neuroprotective agent riluzole, currently in use for the treatment of amyotrophic lateral sclerosis, has been shown to be an activator of TREK-1 and TRAAK channels (Duprat et al., 2000). Volatile general anesthetics such as chloroform and isoflurane have also been shown to target TREK-1 channels (Patel et al., 1999). Further knowledge of the localization and regulation of these channels by cellular and extrinsic signals will be important in targeting specific two-pore channels for therapeutic intervention.

V. Concluding Remarks

K^+ channels are increasingly being elucidated as molecular targets in a number of pathophysiologic states, and they continue to trigger considerable enthusiasm as drug targets. The pivotal role of K^+ channels in various physiological processes including neuronal signaling, vascular and nonvascular muscle contractility, cardiac pacing, auditory function, hormone secretion, immune function, and cell proliferation has been underscored by the recent flurry of discoveries linking K^+ channel mutations to various inherited disorders. Insight into the structure and function of channel proteins coupled with the knowledge of genetic and disease-induced regulation of K^+ channels could undoubtedly improve diagnosis and offer specific candidate genes for the development of appropriate therapies. On the assumption that defined K^+ channel mutations are linked to specific diseases, it may be feasible to conduct a molecular diagnosis to evaluate whether the patient will respond to a drug aimed at specific K^+ channels. It has been shown that differences in K^+ currents may underlie gender-based drug-induced cardiac arrhythmias; for example, women are at far greater risk of torsade de pointes following a variety of drugs including antihistamines, antibiotics, and antiarrhythmic agents (Makkar et al., 1993). Analysis of the differential contribution of K^+ currents contributing to cardiac repolarization could help improve screening methodologies for individuals at risk for

drug-induced arrhythmias and direct development of drugs with reduced incidence of inducing arrhythmias. Knowledge of specific mutations may also lead to validation of more suitable animal models of disease to help preclinical assessment of novel compounds. In the coming years, modulating K^+ channel gene expression in diseased tissues via various gene delivery approaches or antisense oligonucleotides could present an additional avenue to treat various diseases and/or, in combination with pharmacotherapy, to enhance the selectivity of K^+ channel modulators. Additionally, unraveling precise in situ channel combinations, localization, and channel regulation in disease pathologies could shed light on developing better therapeutic strategies. Targeting diverse auxiliary subunits or modulating the interactions of auxiliary subunits with the pore-forming subunit may also provide alternate avenues for identifying selective regulators of K^+ channel function. It is to be anticipated that these efforts could collectively enhance the development of selective compounds that modulate the various classes of K^+ channels with promising therapeutic and prophylactic utility.

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